

Figure 1A: Purification of bacterially produced E7 via MonoQ-column

15% PAGE

-1 +1
 sup. UC
 pellet UC
 precip. sup.
 column input
 Fraction 11
 Fraction 12
 Fraction 14
 Fraction 15
 Fraction 17
 Fraction 18
 Fraction 19
 Fraction 20
 Fraction 21
 Fraction 22
 Fraction 23
 SDS-7-Marker

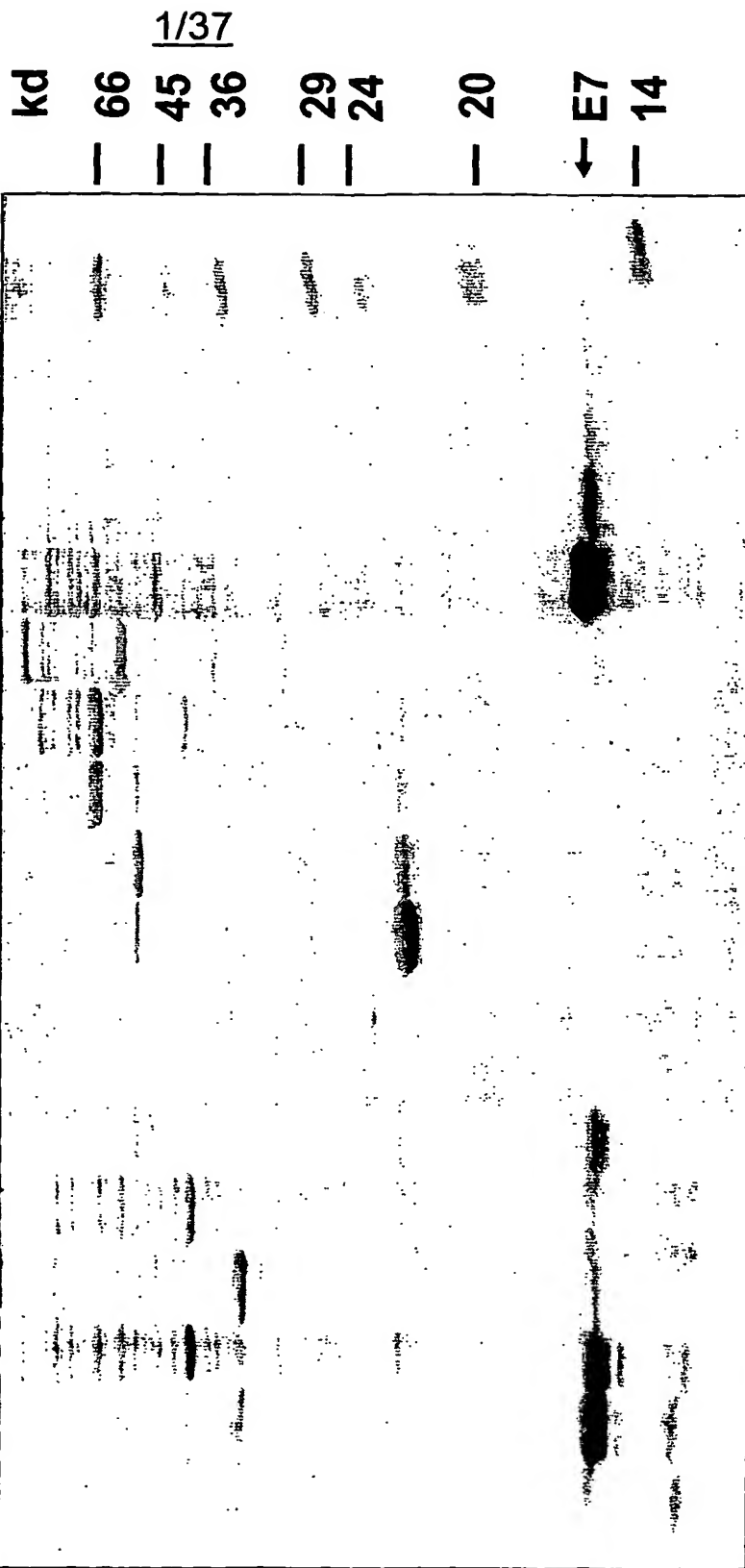
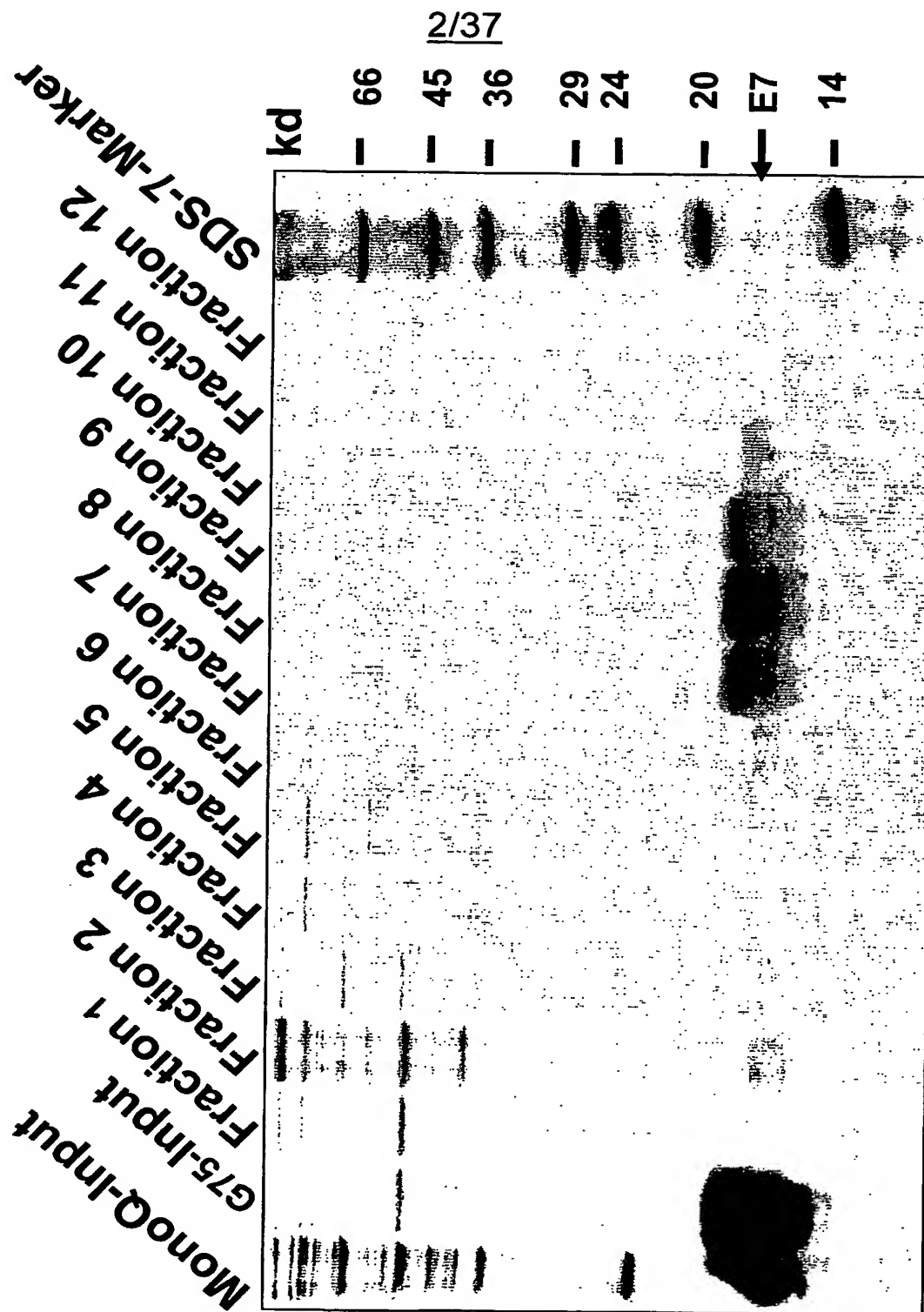


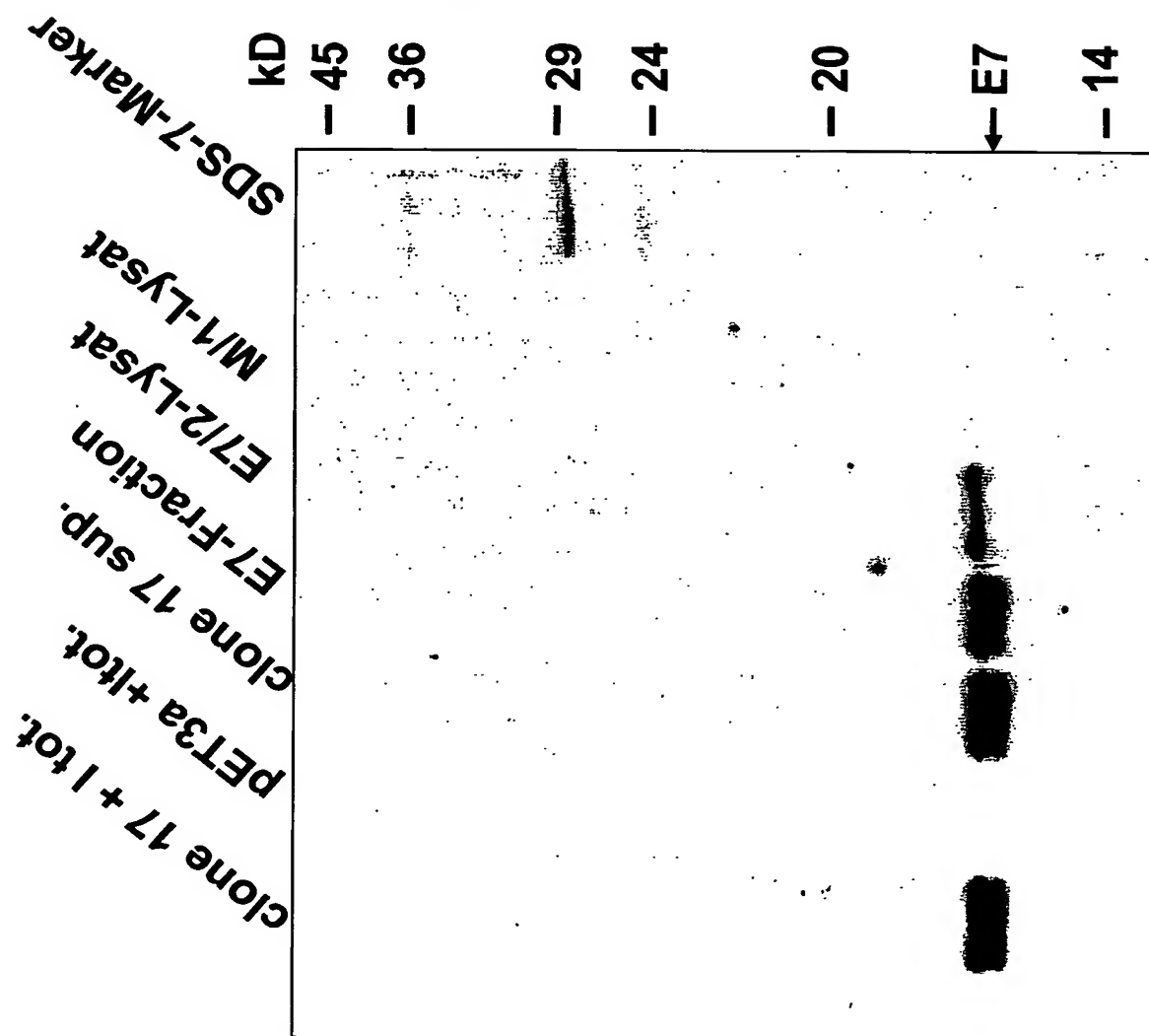
Figure 1B: G75 – Gelfiltration, 15% PAGE



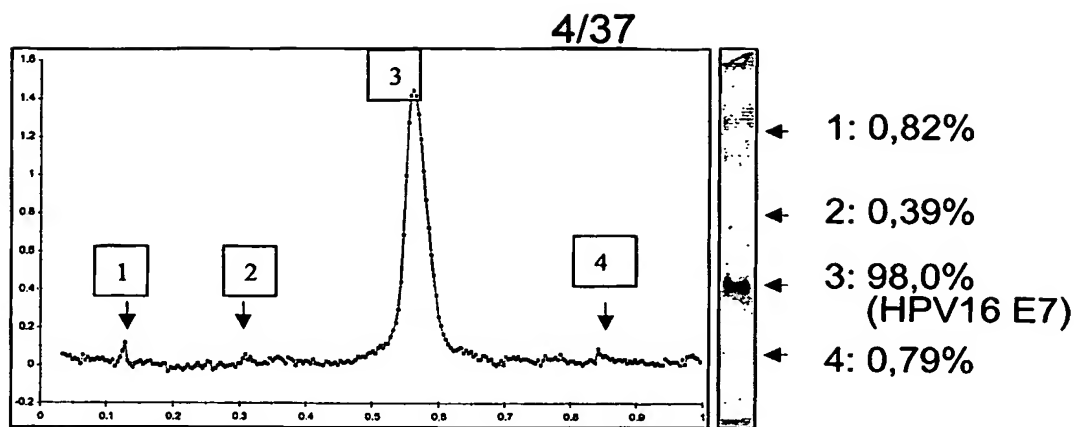
BEST AVAILABLE COPY

3/37

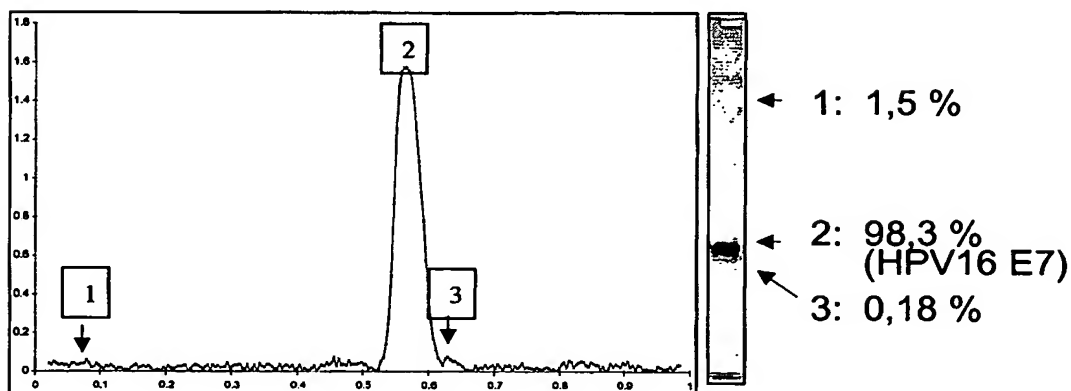
Figure 1C:
Western Blot,
Santa Cruz (sc 6981)
monoclonal anti-E7
antibody 1:5000



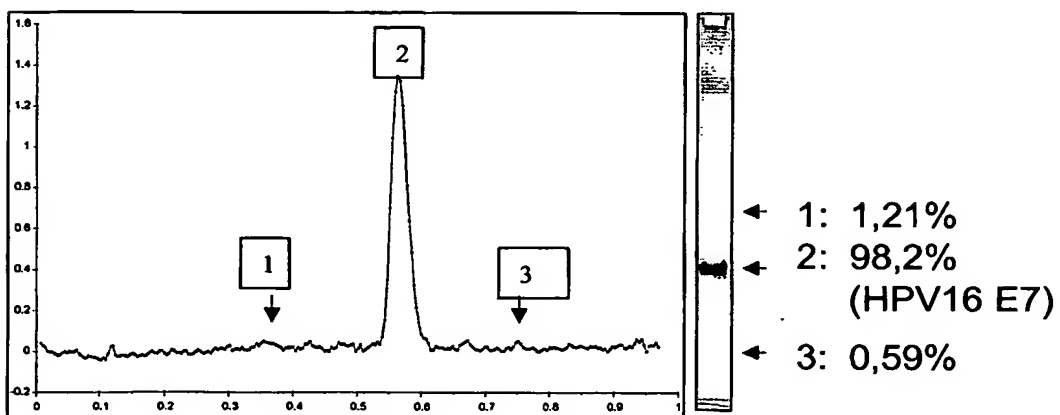
BEST AVAILABLE COPY



E7 preparation A



E7 preparation B



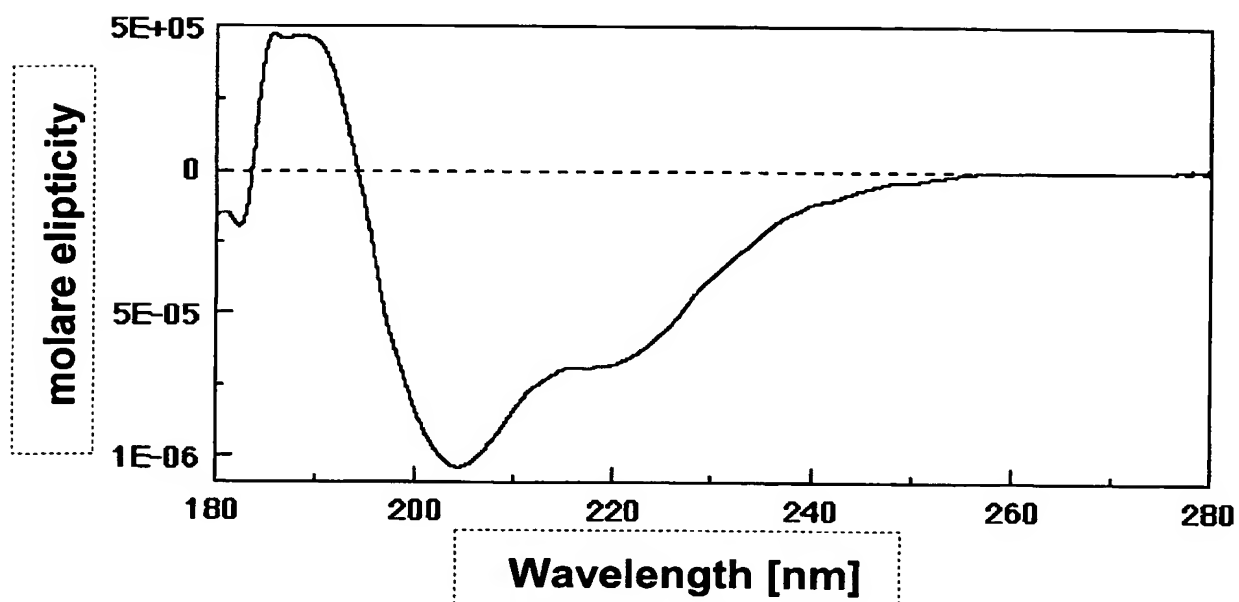
E7 preparation C

Figure 1D

BEST AVAILABLE COPY

5/37

Figure 1E
Circular dichroism spectroscopy measurements:
Secondary structures in HPV16 E7 protein



structure	
α -helices	7 - 8 %
β -sheets	45 - 47 %
turn	3 - 5 %
coils	40 - 43 %

BEST AVAILABLE COPY

Figure 2A:
Westernblot rabbit anti-HPV-16 E7 antibody (14/3)

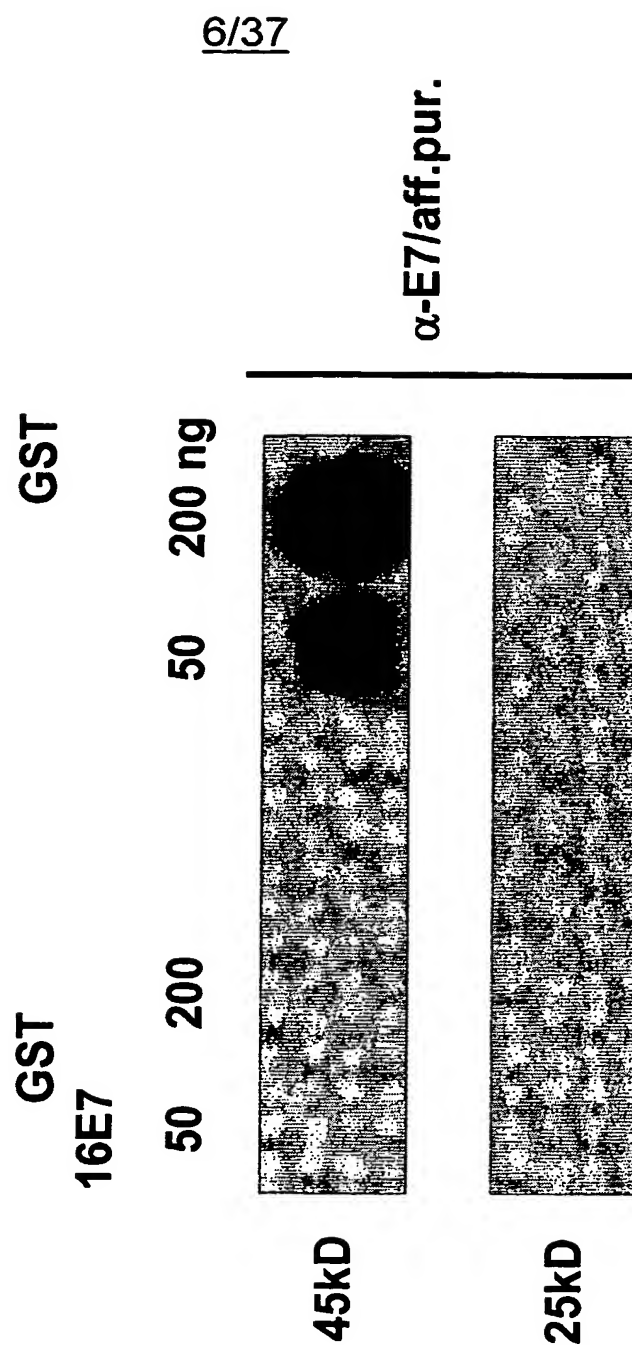
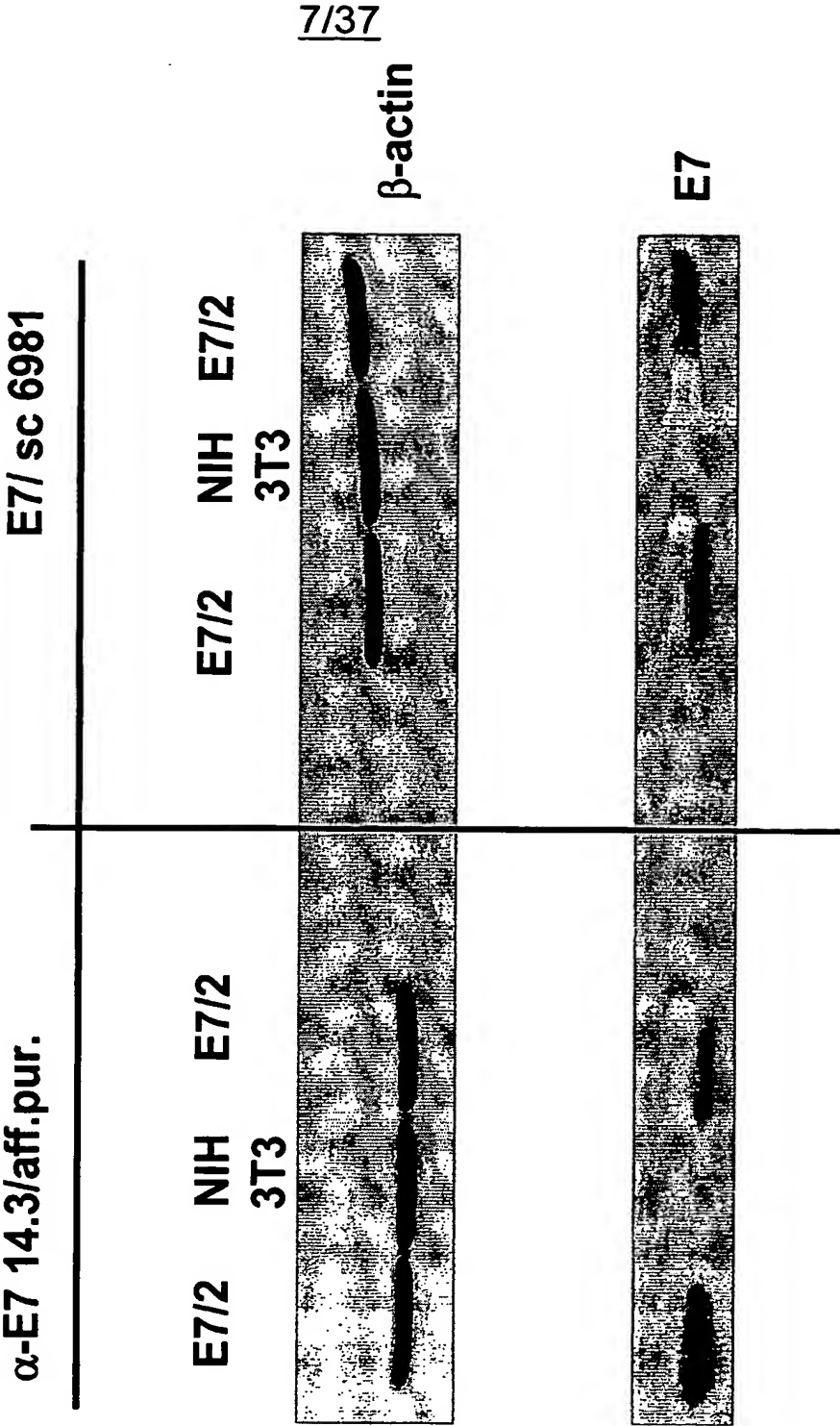
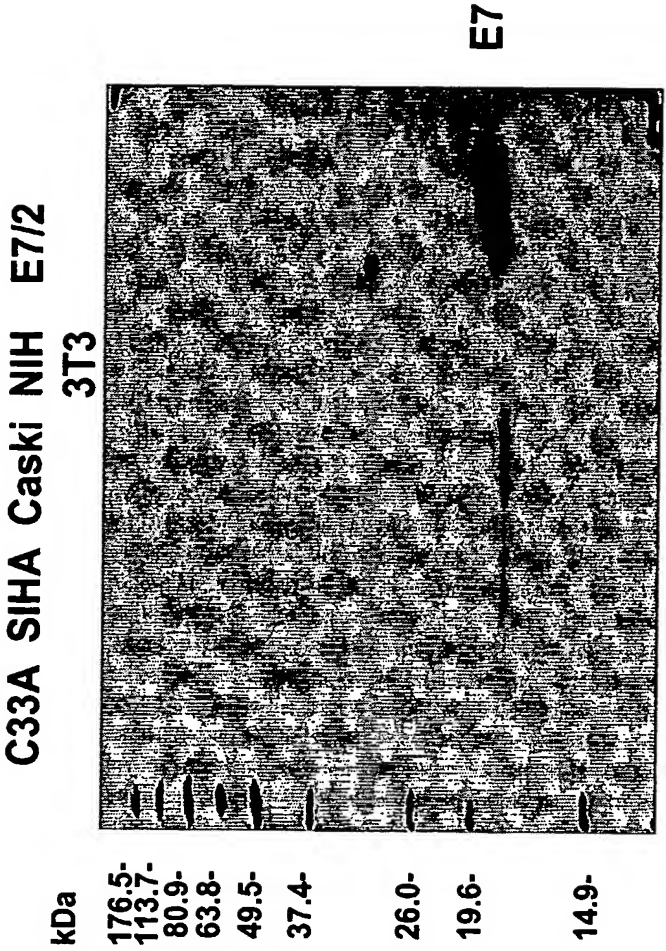


Figure 2B: Westernblot rabbit anti-HPV-16 E7 antibody versus monoclonal anti-HPV-16 E7 antibody Santa Cruz (sc 6981)



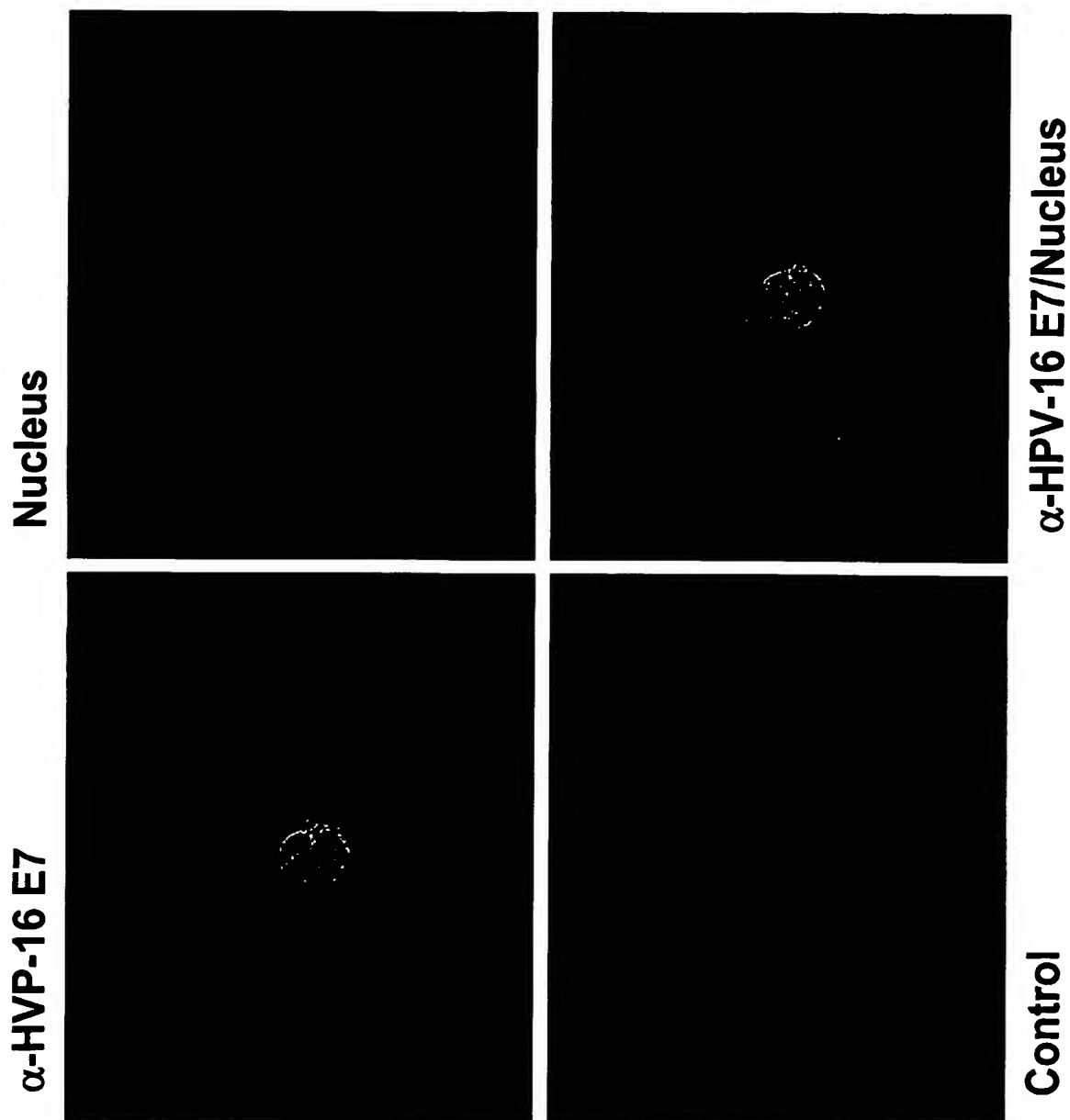
8/37

Figure 2C:
Westernblot affinity purified goat anti-HPV-16 E7 antibody,
HPV-16 E7 detection in cell lysates



9/37

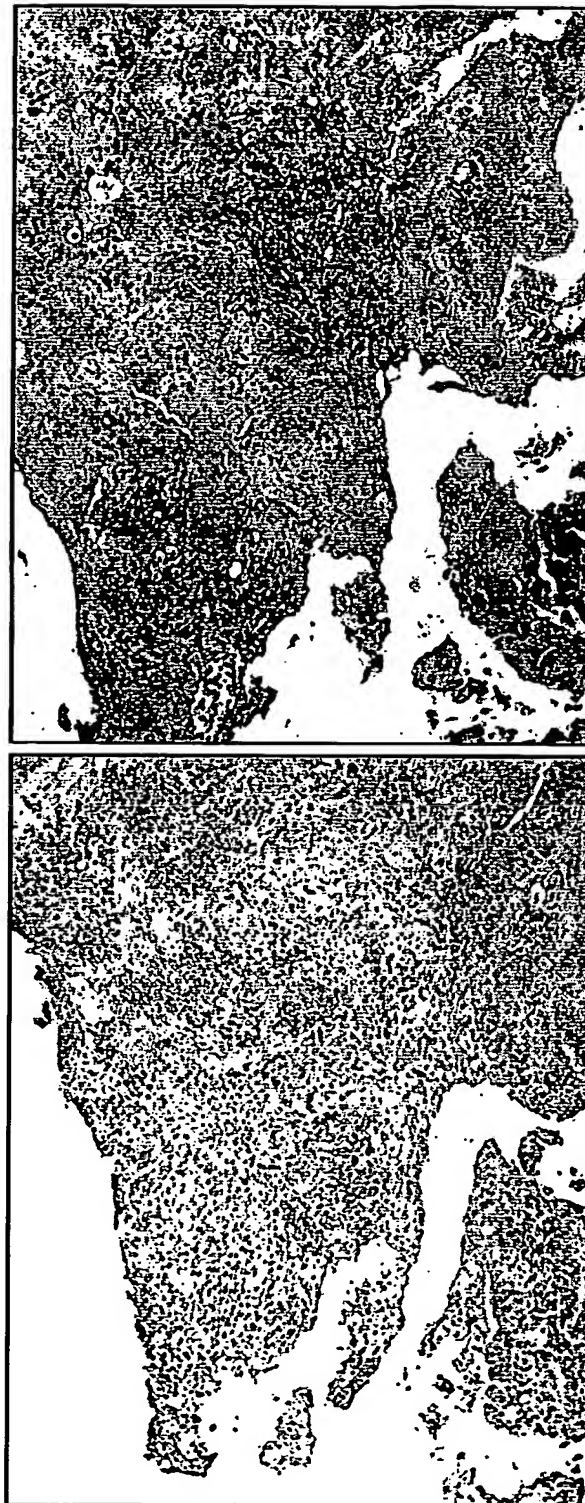
Figure 3
U-2-OS cells transiently transfected with
pX-HPV-16 E7



BEST AVAILABLE COPY

10/37

Figure 4A
Paraffin section: HPV-16 DNA positive cervical carcinoma
Control Preimmunserum (left), rabbit anti-HPV-16 E7 antibodies



BEST AVAILABLE COPY

11/37

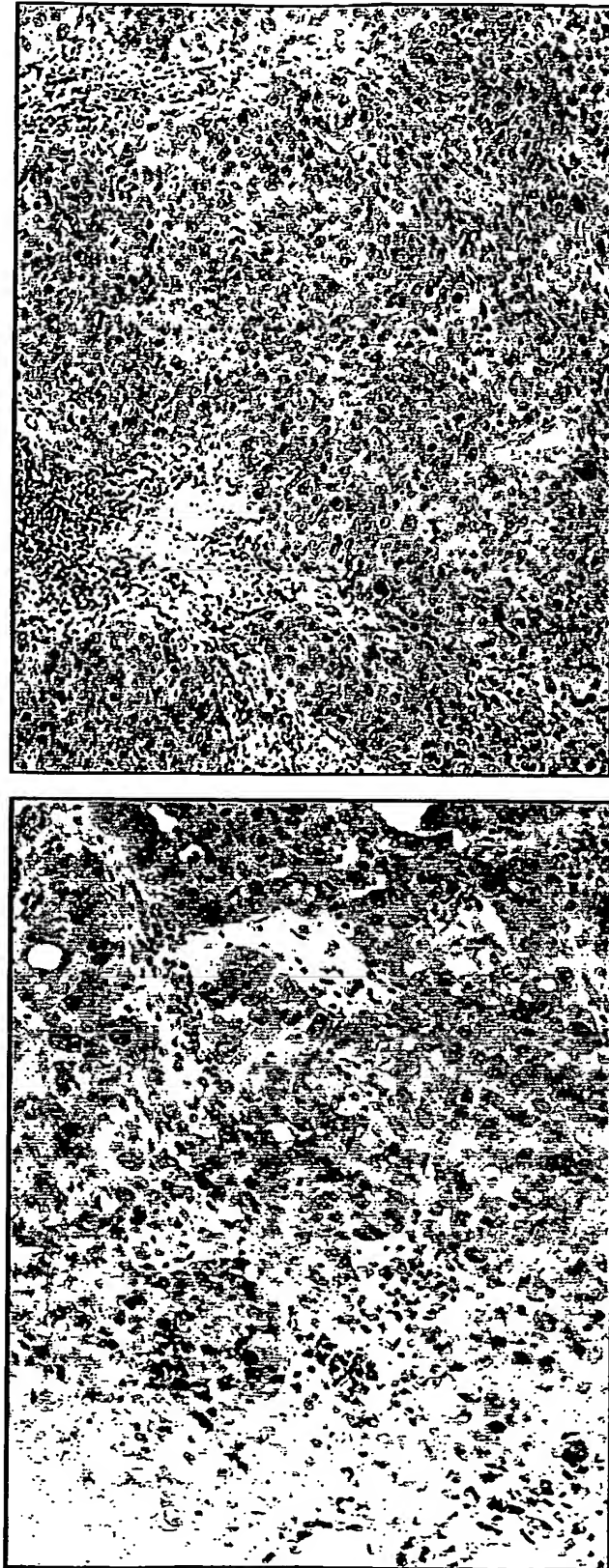
Figure 4B
Paraffin section stained with rabbit anti-HPV-16 E7 antibodies
Negative control normal squamous epithelium (left), no first antibody (right)



BEST AVAILABLE COPY

12/37

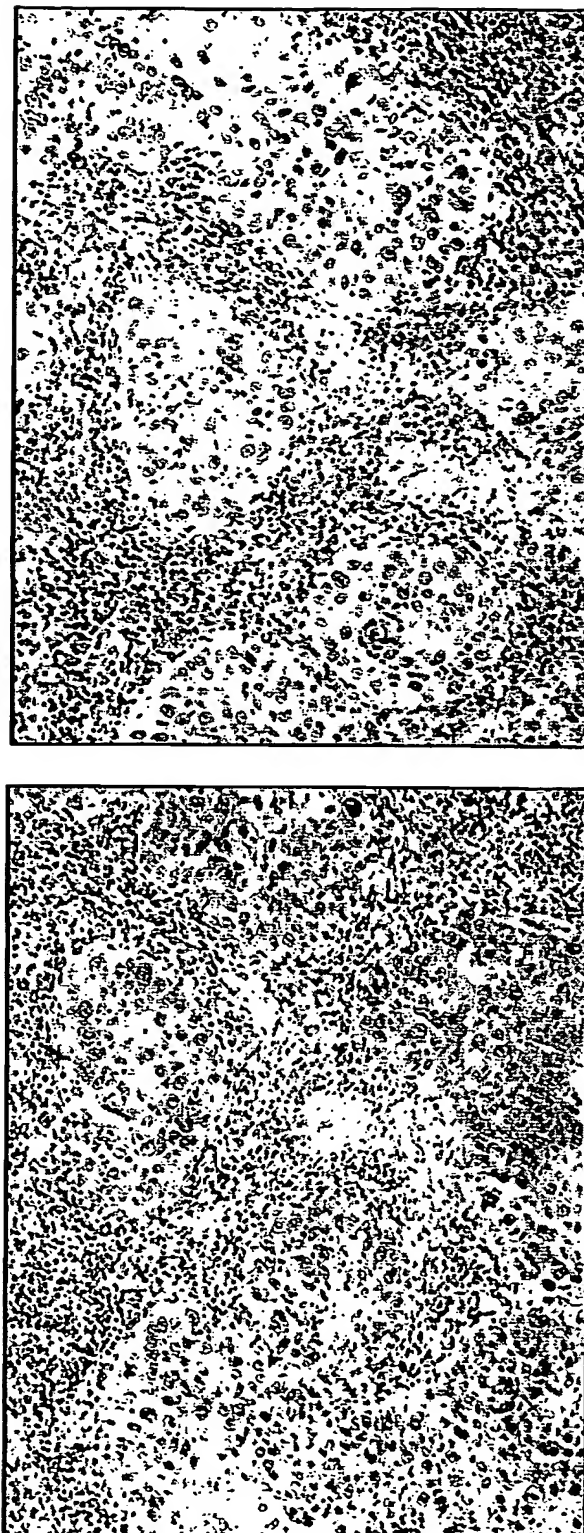
Figure 4C
Paraffin section: HPV-16 DNA positive cervical carcinoma
stained with rabbit anti-HPV-16 E7 antibodies



BEST AVAILABLE COPY

13/37

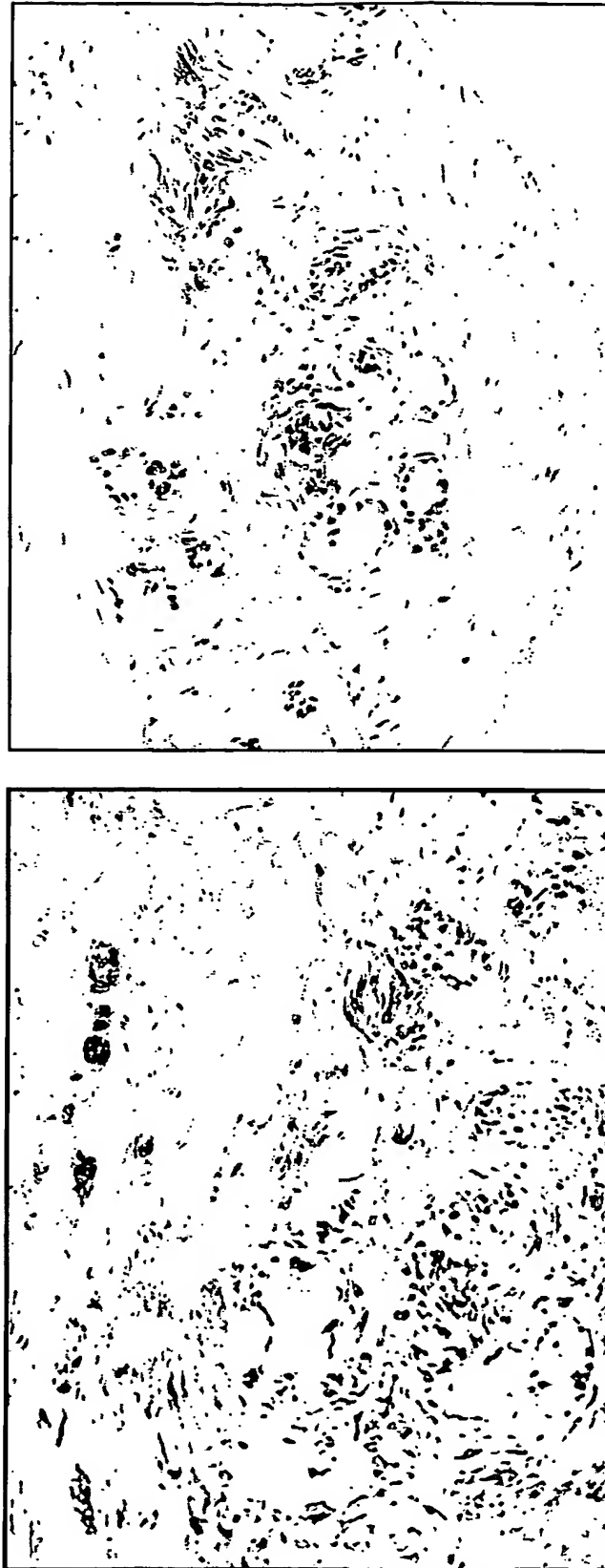
Figure 4D
Paraffin section: HPV-16 DNA positive cervical carcinoma
rabbit anti-HPV-16 E7 (left) /Competition 1µg/µl 16 E7 (right)



BEST AVAILABLE COPY

14/37

Figure 5
E7-positive prostate carcinoma (left)
E7-negative prostate carcinoma (right)



BEST AVAILABLE COPY

15/37



Figure 6

BEST AVAILABLE COPY

16/37

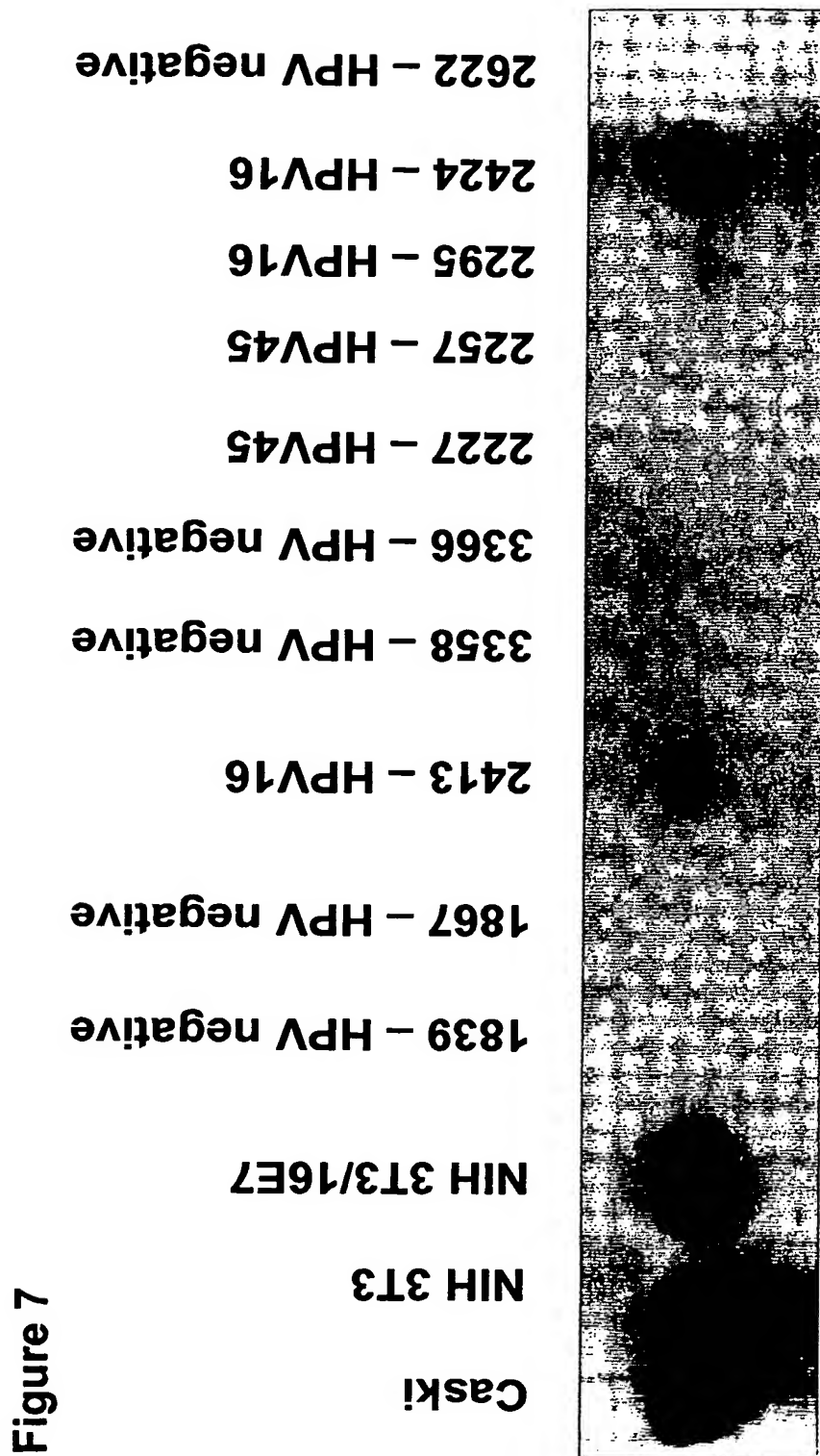
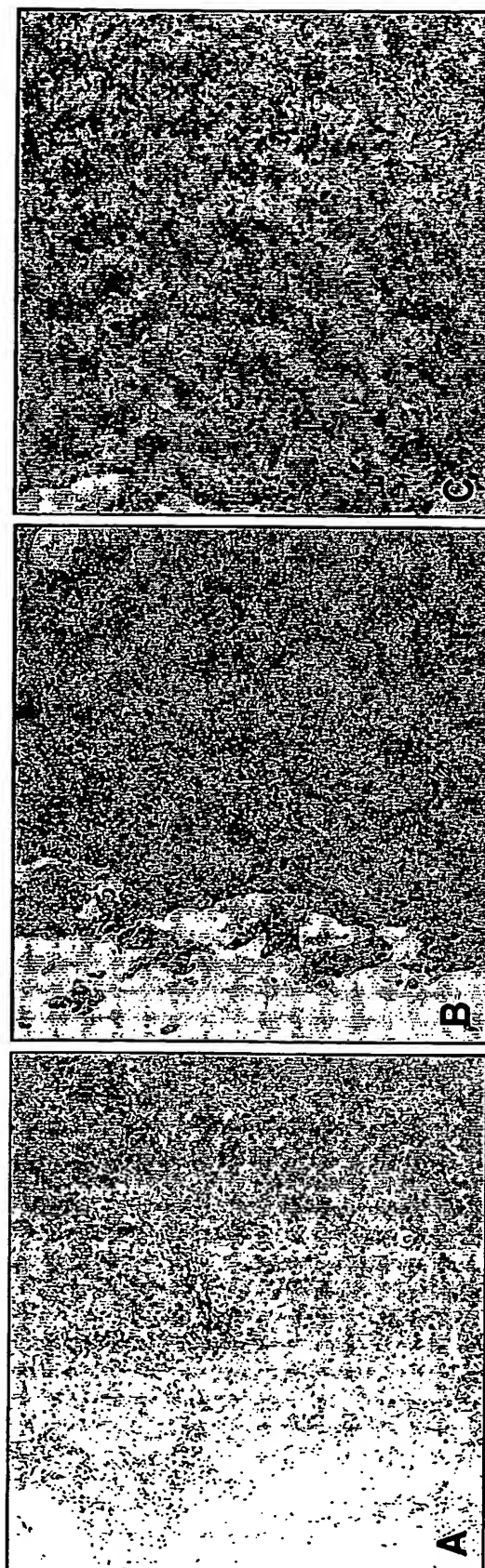


Figure 7

BEST AVAILABLE COPY

17/37

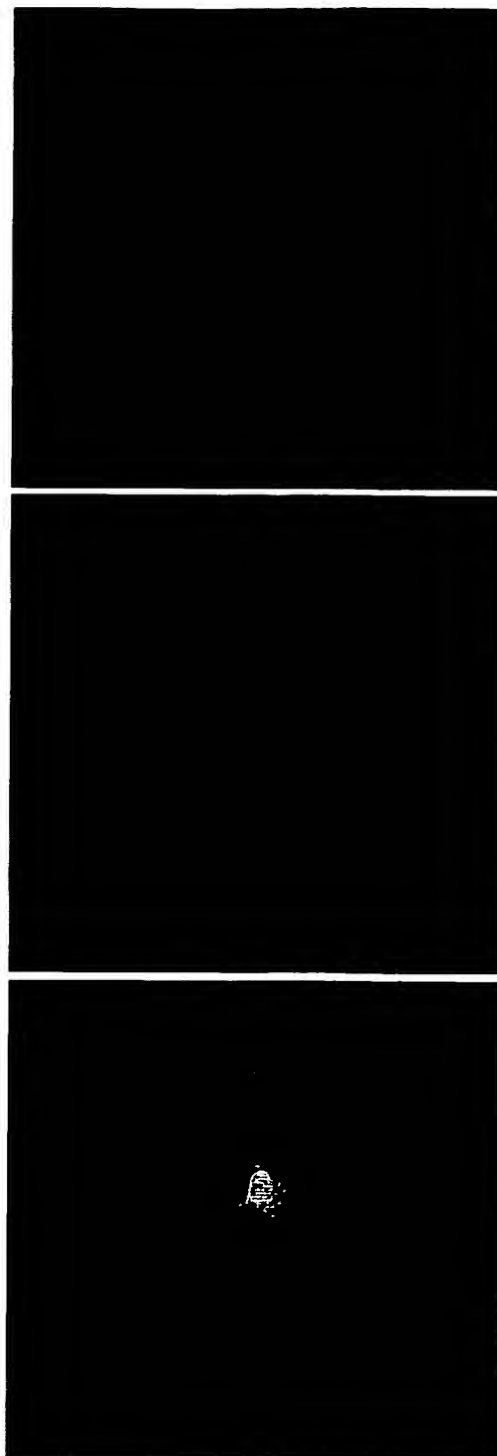
Figure 8
Immunohistochemical staining of HPV-16 E7 protein in paraffin embedded cervical carcinoma tissue sections ;
A: anti HPV-16 E7 antibody described herein;
B: monoclonal anti-HPV-16 E7 antibody Santa Cruz, clone ED17;
C: monoclonal anti-HPV-16 E7 antibody Zymed, clone 8C9X.



BEST AVAILABLE COPY

Figure 9
U-2-OS cells transiently transfected with pX-HPV-16 E7

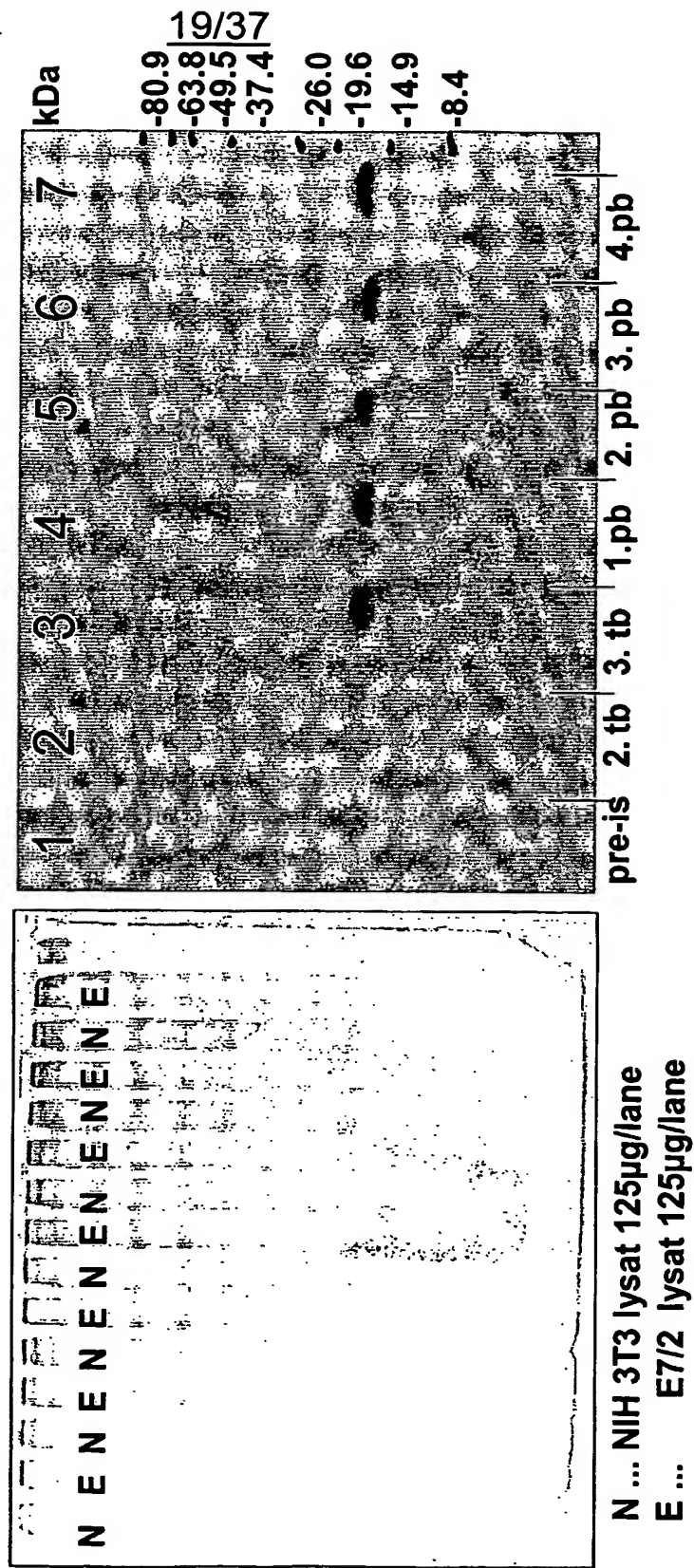
rabbit anti-HVP-16 E7 anti-HVP-16 E7 anti-HVP-16 E7
ab described herein Cruz, clone ED17 Zymed, clone 8C9X



18/37

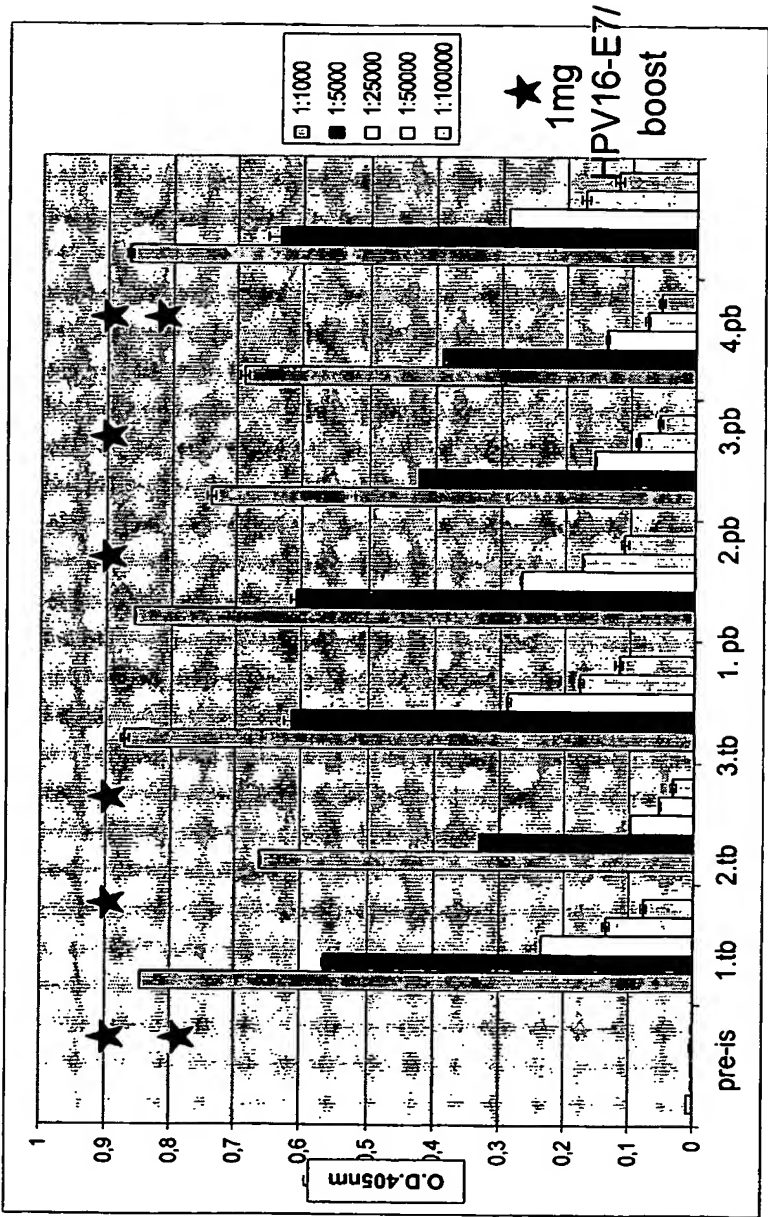
BEST AVAILABLE COPY

Figure 10 :
Western-blot as quality control of goat-HPV16-E7 antiserum



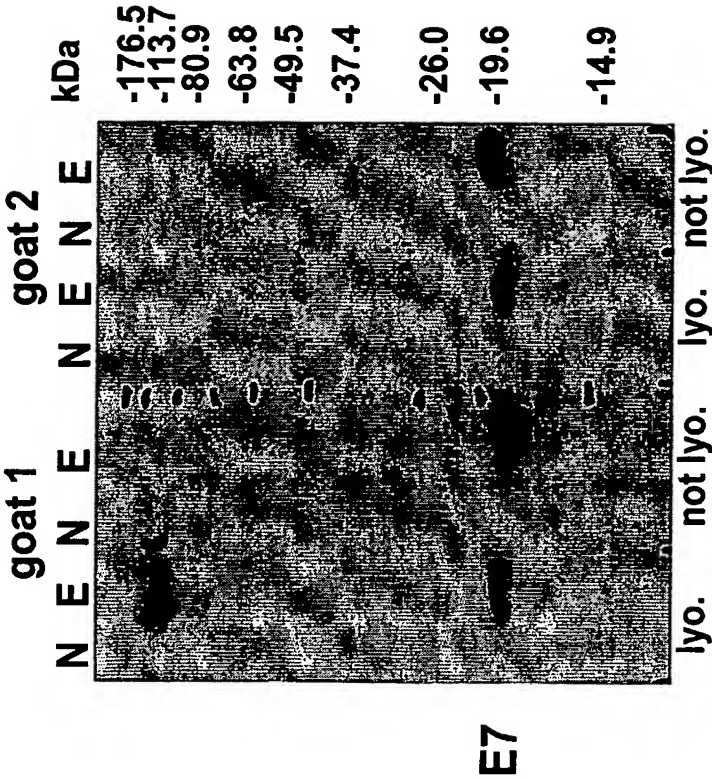
20/37

Figure 11 :
Direct ELISA to determine the titer of goat HPV16-E7 antiserum



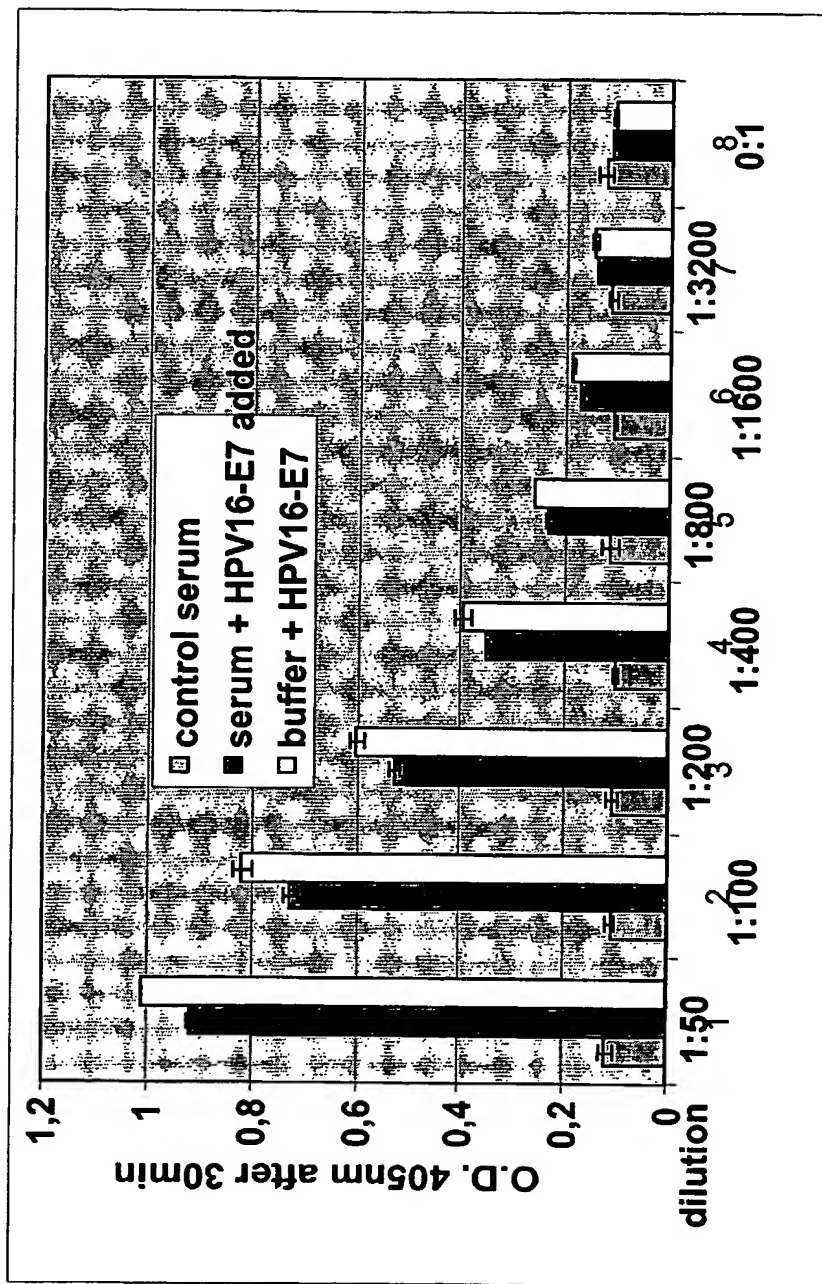
21/37

Figure 12 :
Western-blot as quality control for purified, reconstituted
anti HPV16-E7 antibody from 2 goats



22/37

Figure 13 :
Direct sandwich ELISA to detect HPV16-E7 in serum samples



BEST AVAILABLE COPY

Figure 14 :
Direct sandwich ELISA to detect HPV16-E7 in patient serum samples

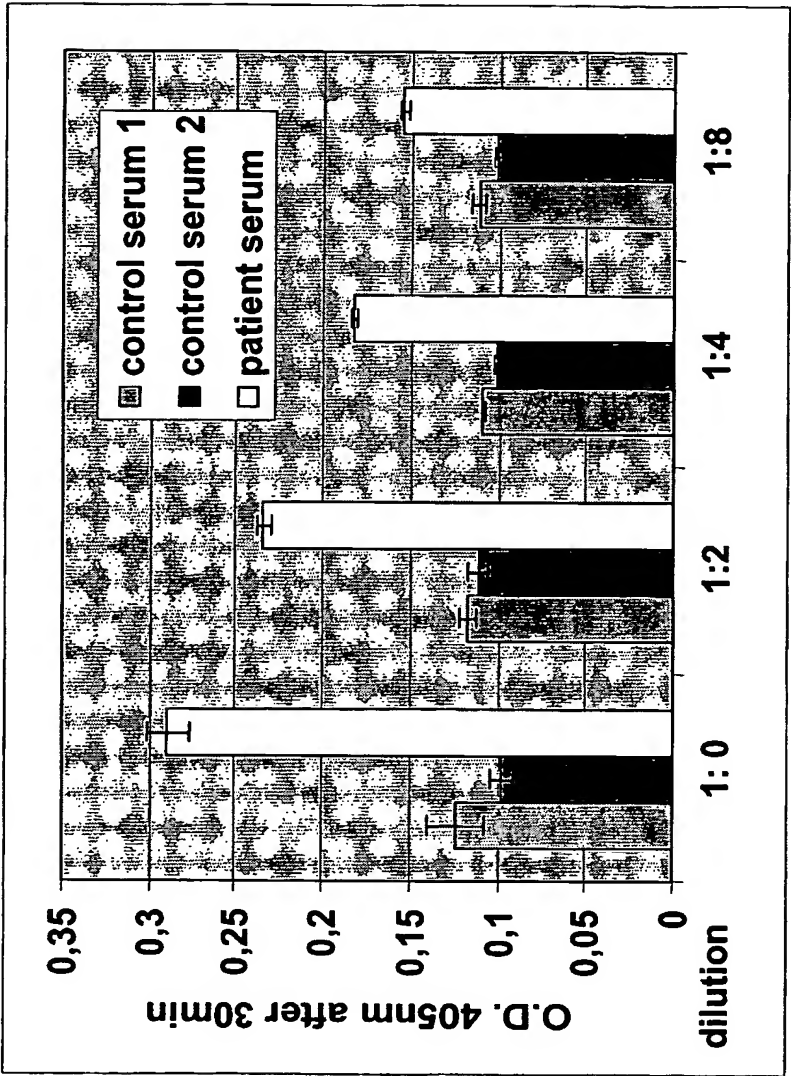


Figure 15 : Immunofluorescence detection of different high risk HPV E7 proteins by anti HPV-16 E7 antibodies from goat

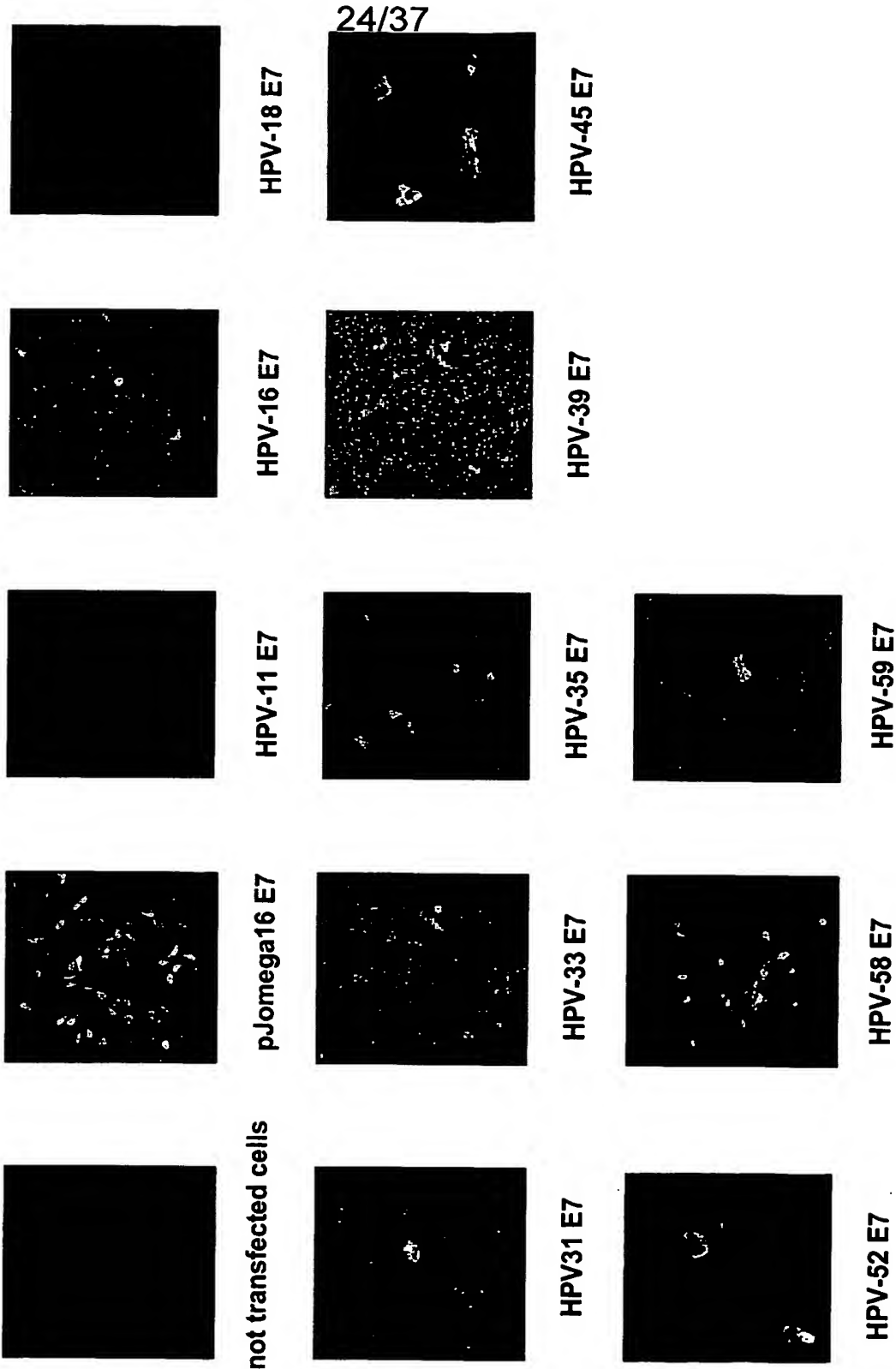
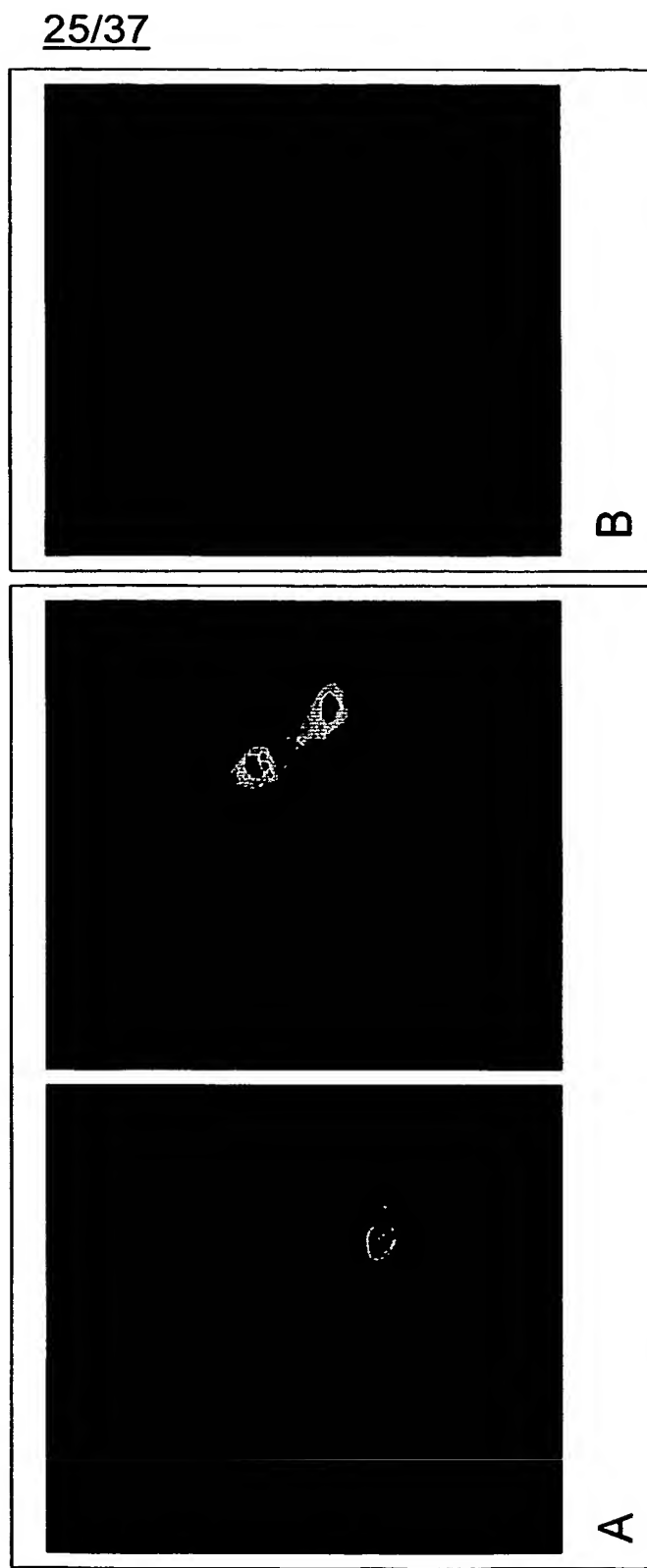


Figure 16
Competition of specific antibody activity from affinity purified
Amynon goat anti HPV16-E7 pAB:



BEST AVAILABLE COPY

Figure 17:
Purification of recombinant HPV18-E7, expressed in *E.coli*; all SDS-
PAGE 12.5% under reducing conditions

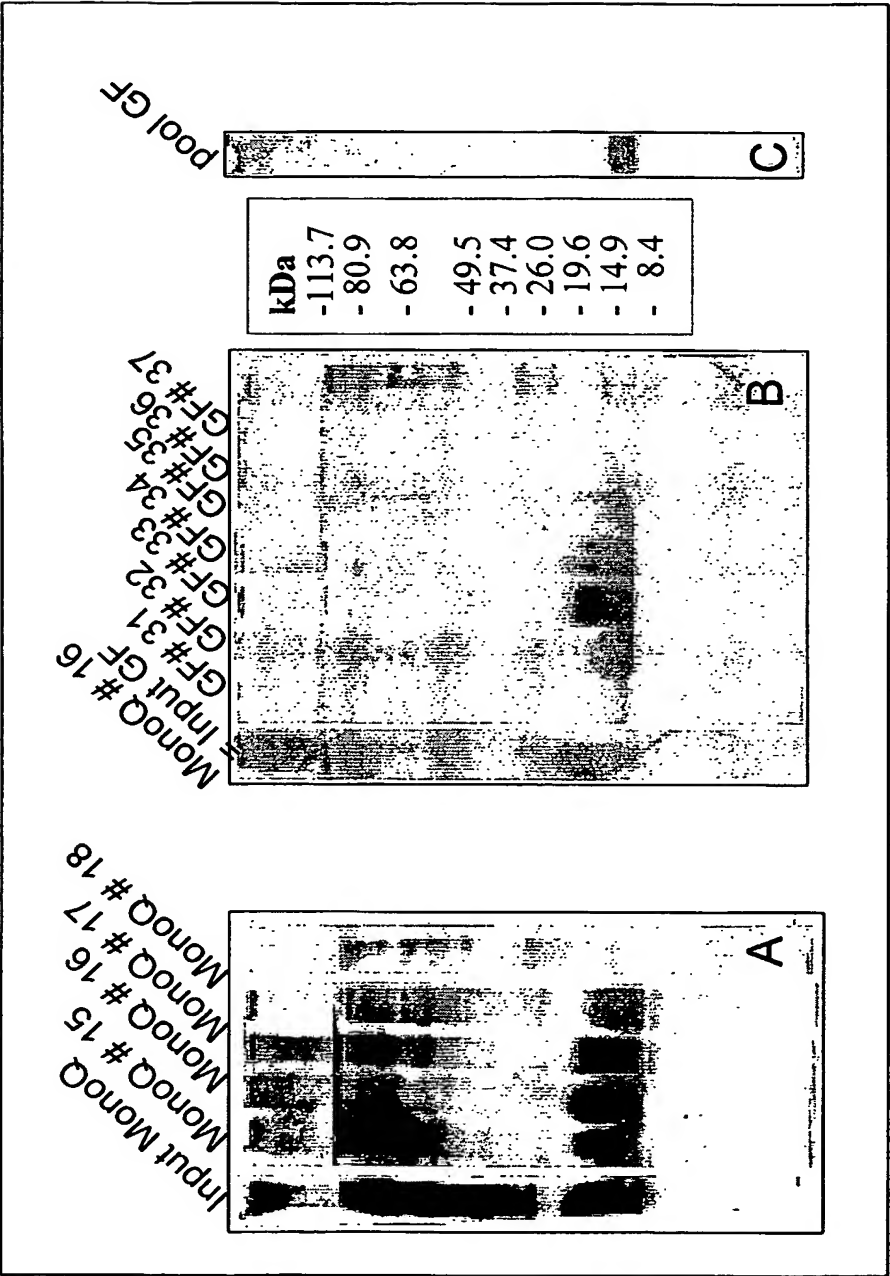
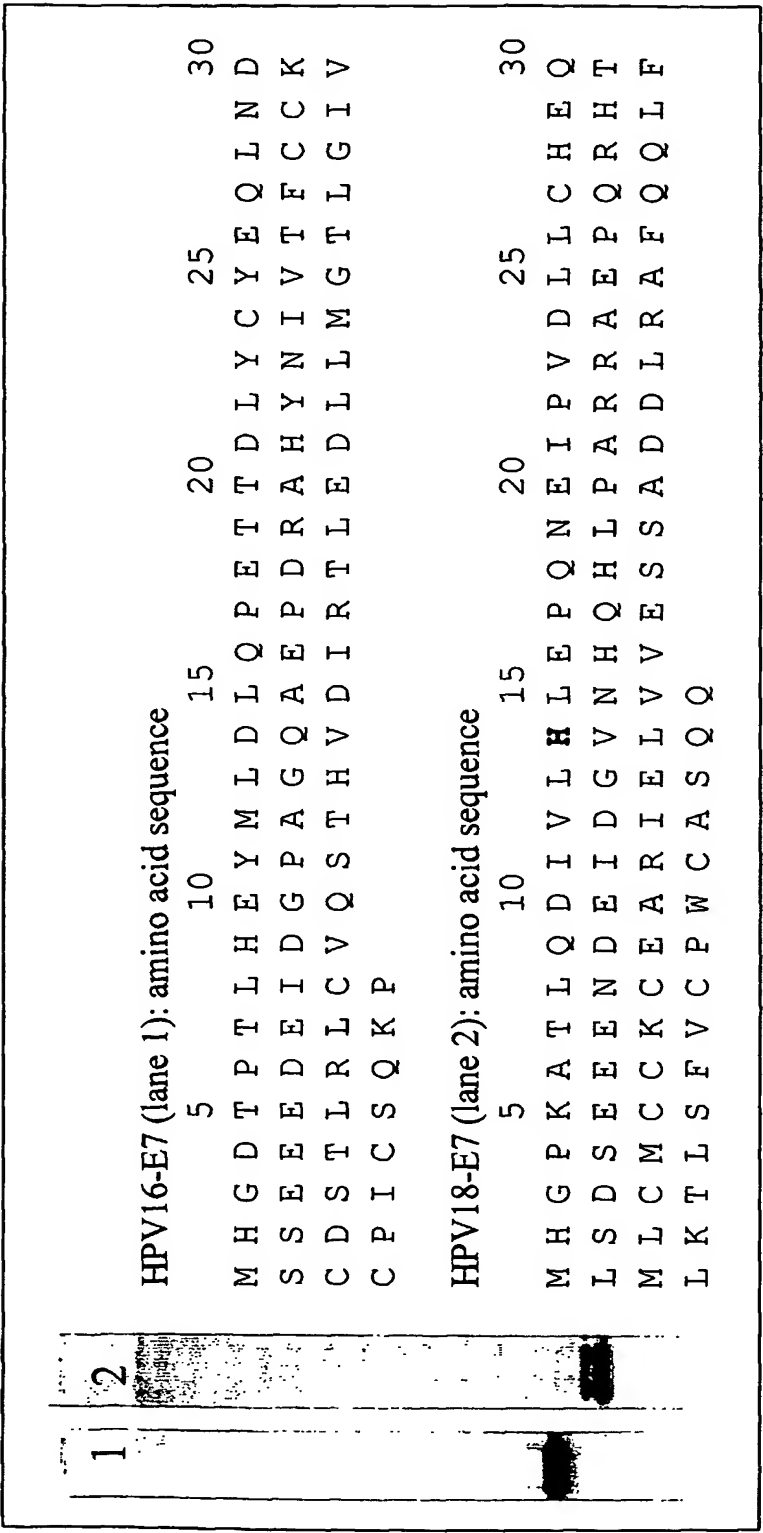
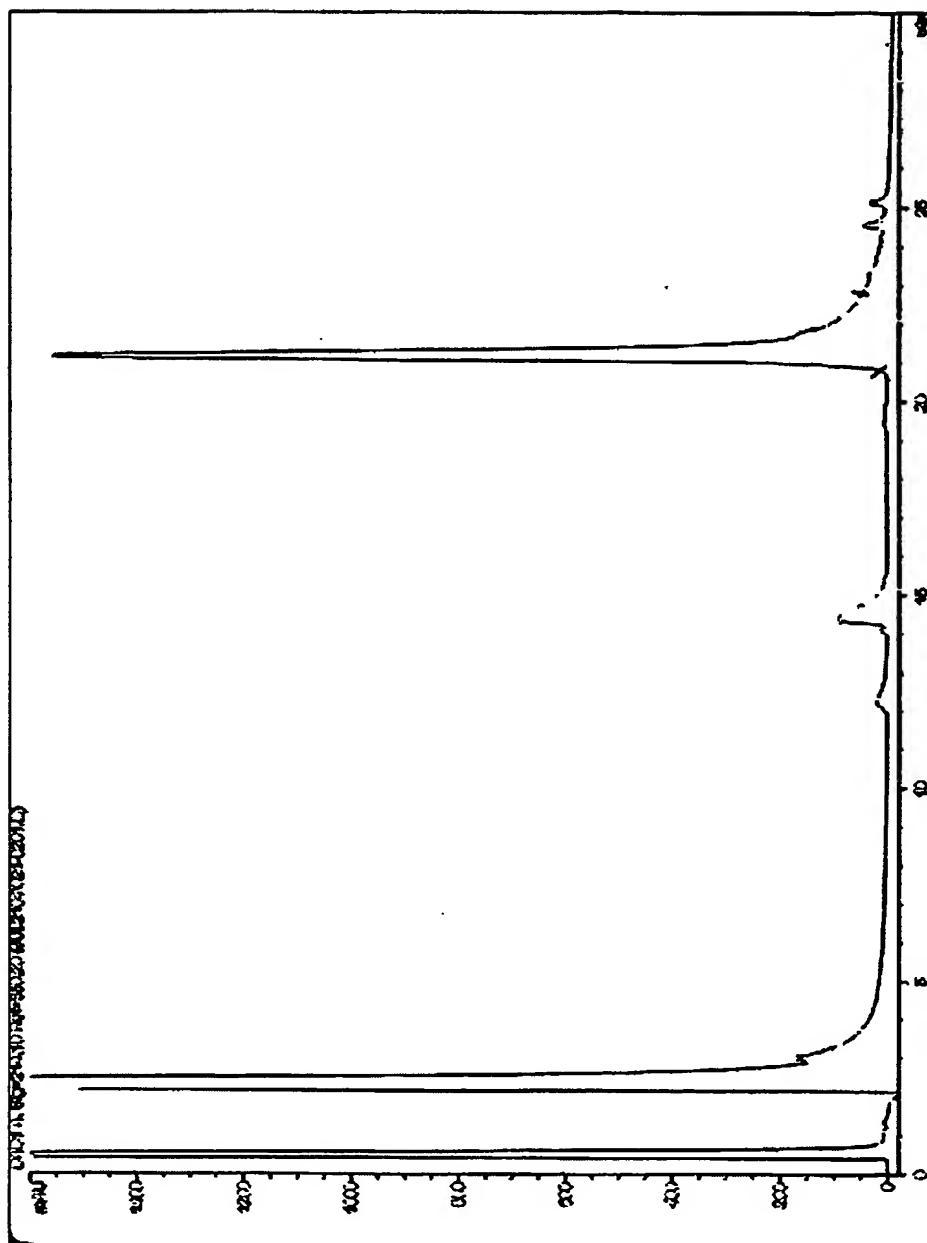


Figure 18A:
Purified HPV16-E7 (lane 1) and HPV18-E7 (lane 2) protein that was
used for immunisations.



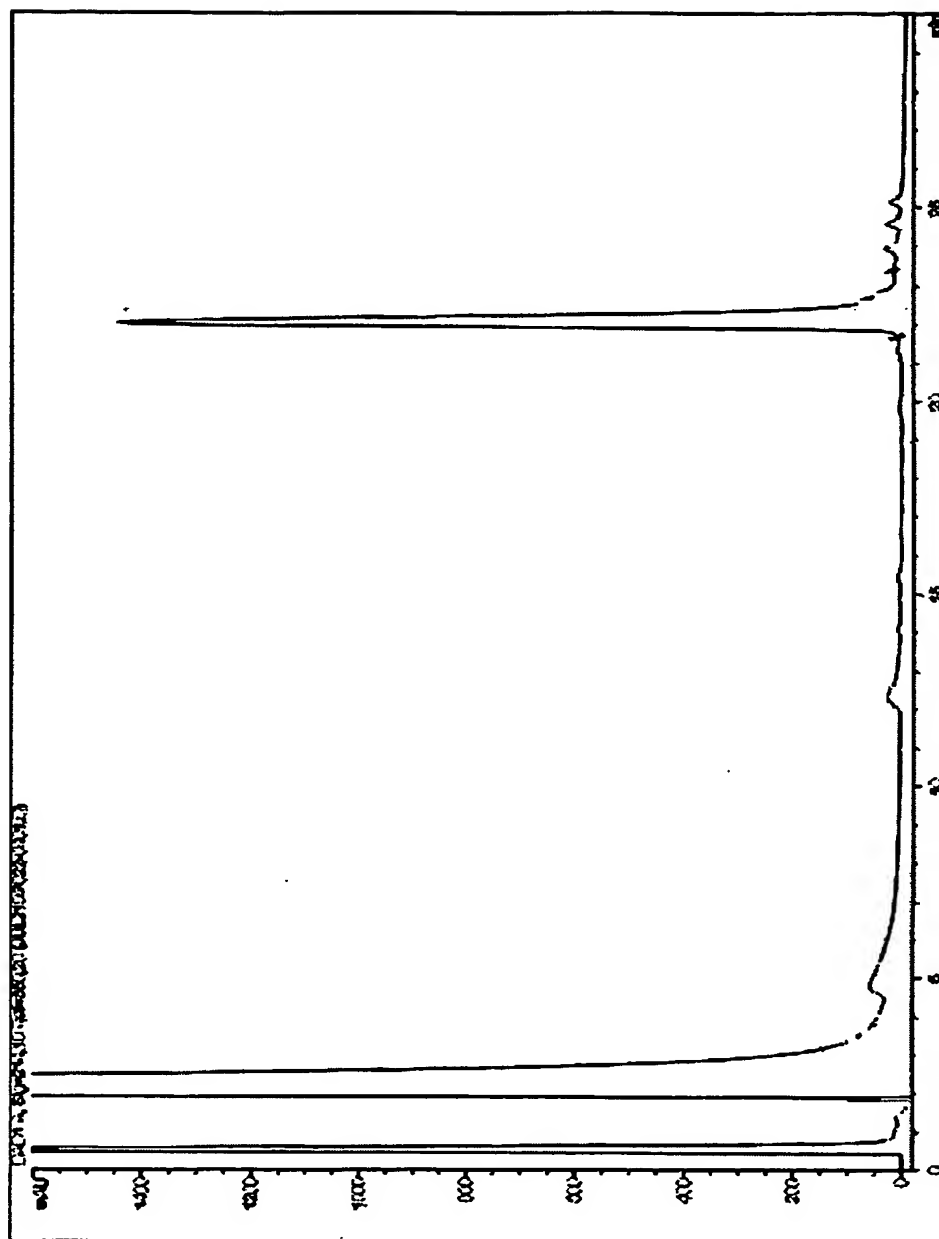
28/37

Figure 18B :
Purified HPV16-E7 protein after reduction and pyridylethylation
prior to tryptic digest.



BEST AVAILABLE COPY

Figure 18C :
Purified HPV18-E7 protein after reduction and pyridylethylation
prior to tryptic digest.



BEST AVAILABLE COPY

30/37

Figure 19 :
Test of immune responses after immunisation of rabbit (left) and goat
(right) with HPV18-E7 protein.

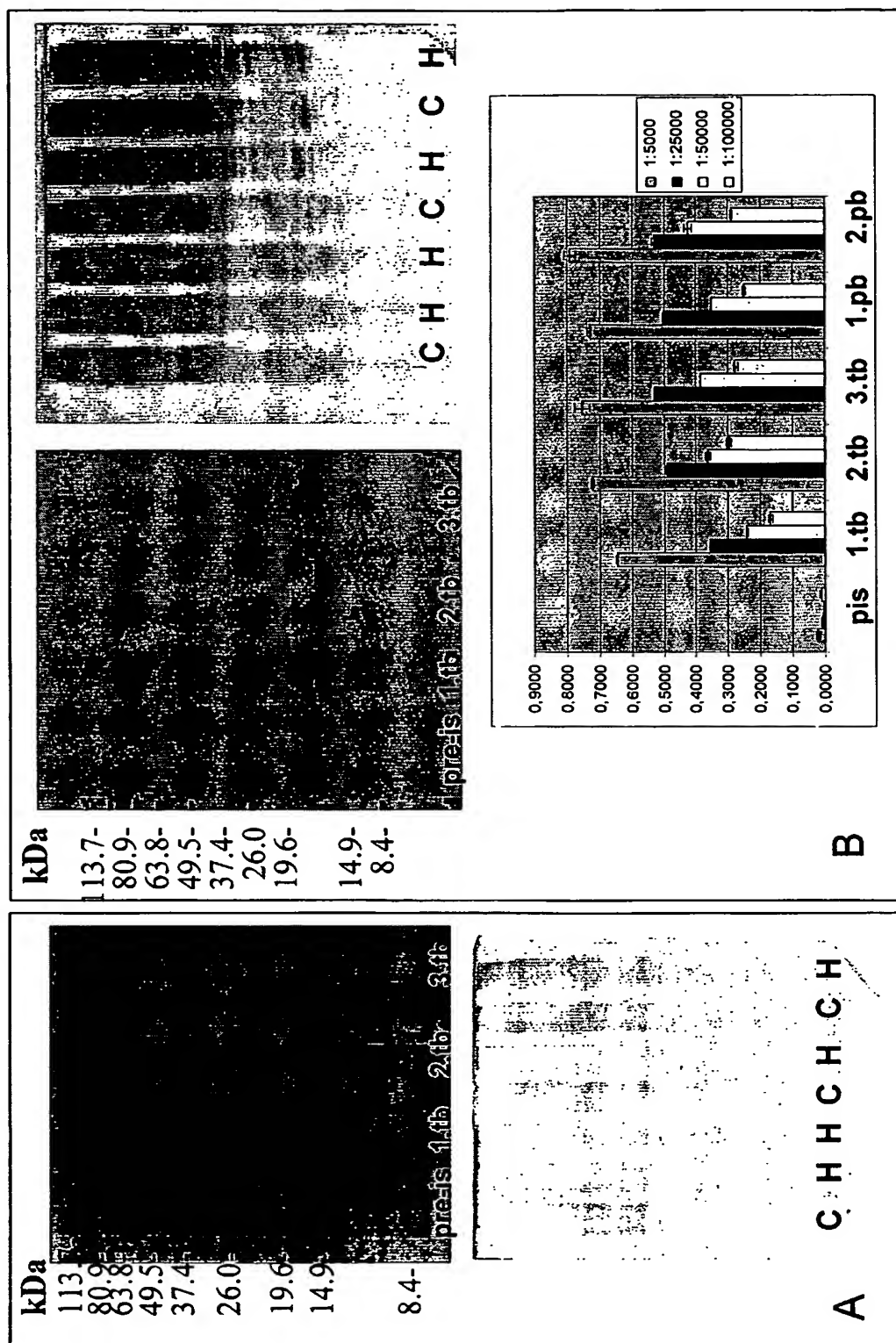


Figure 20A:
ELISA performed with serum from mice (O, L, R, RL, RRL) immunised with HPV16-E7 protein.

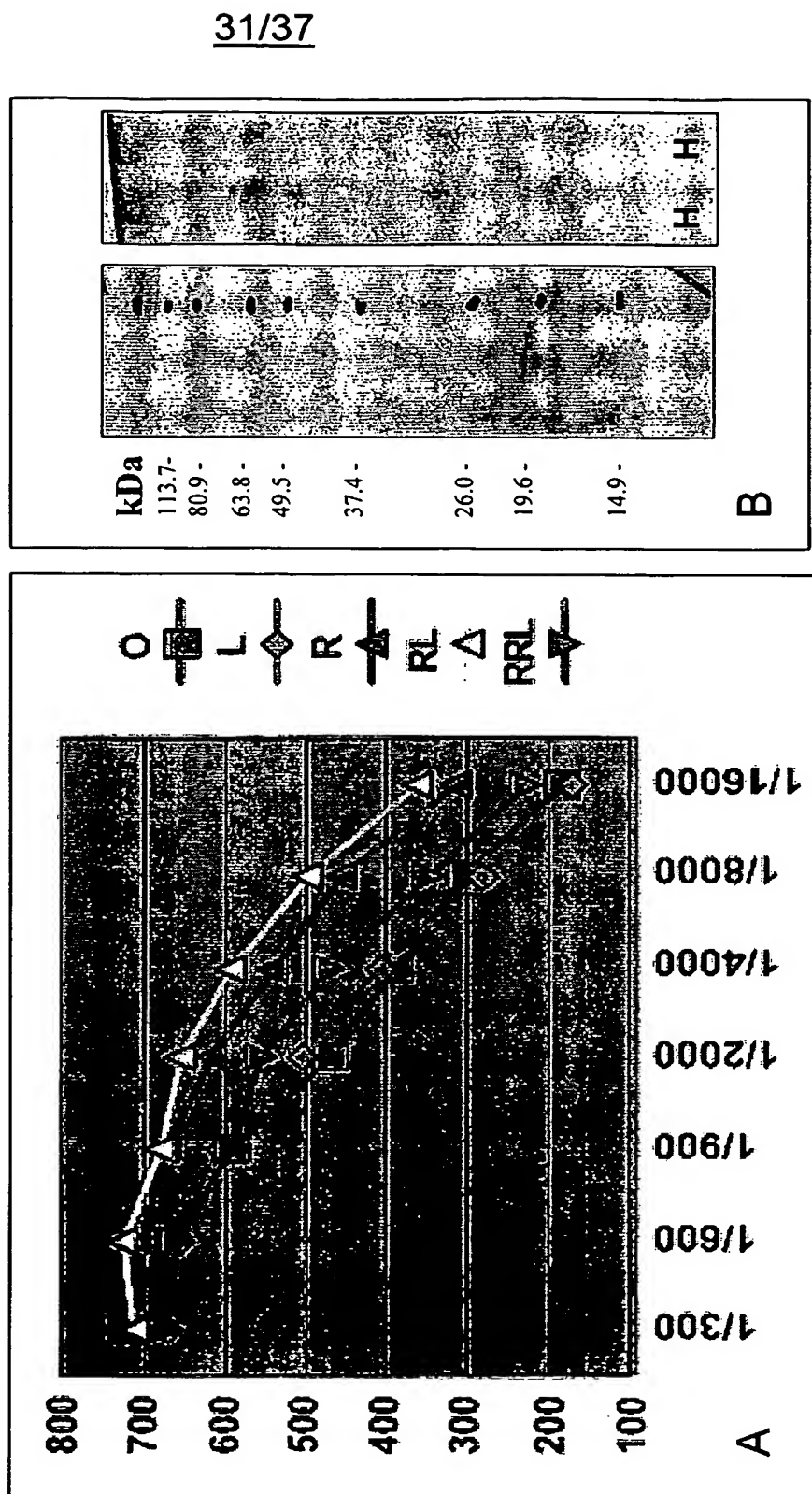
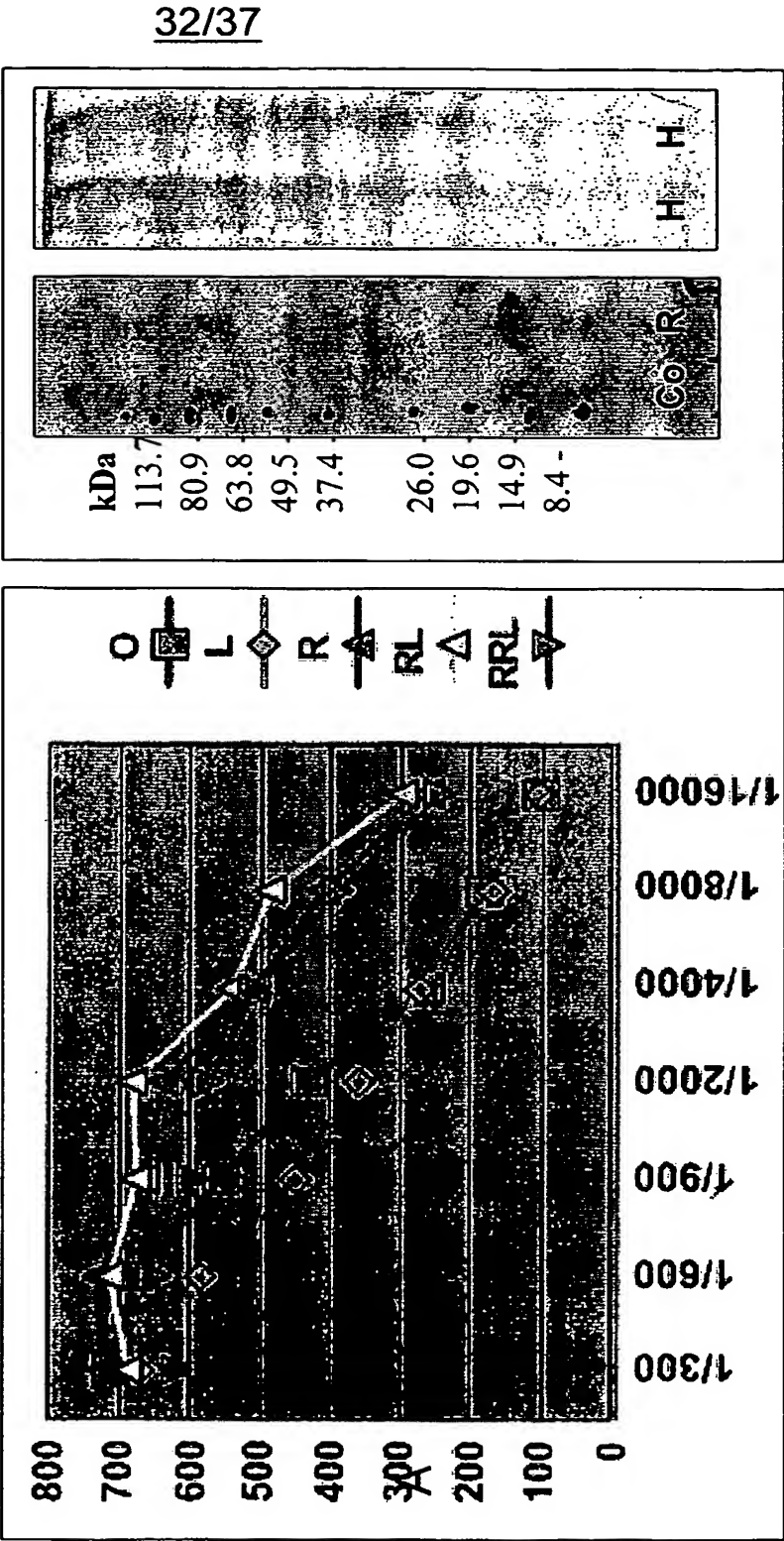
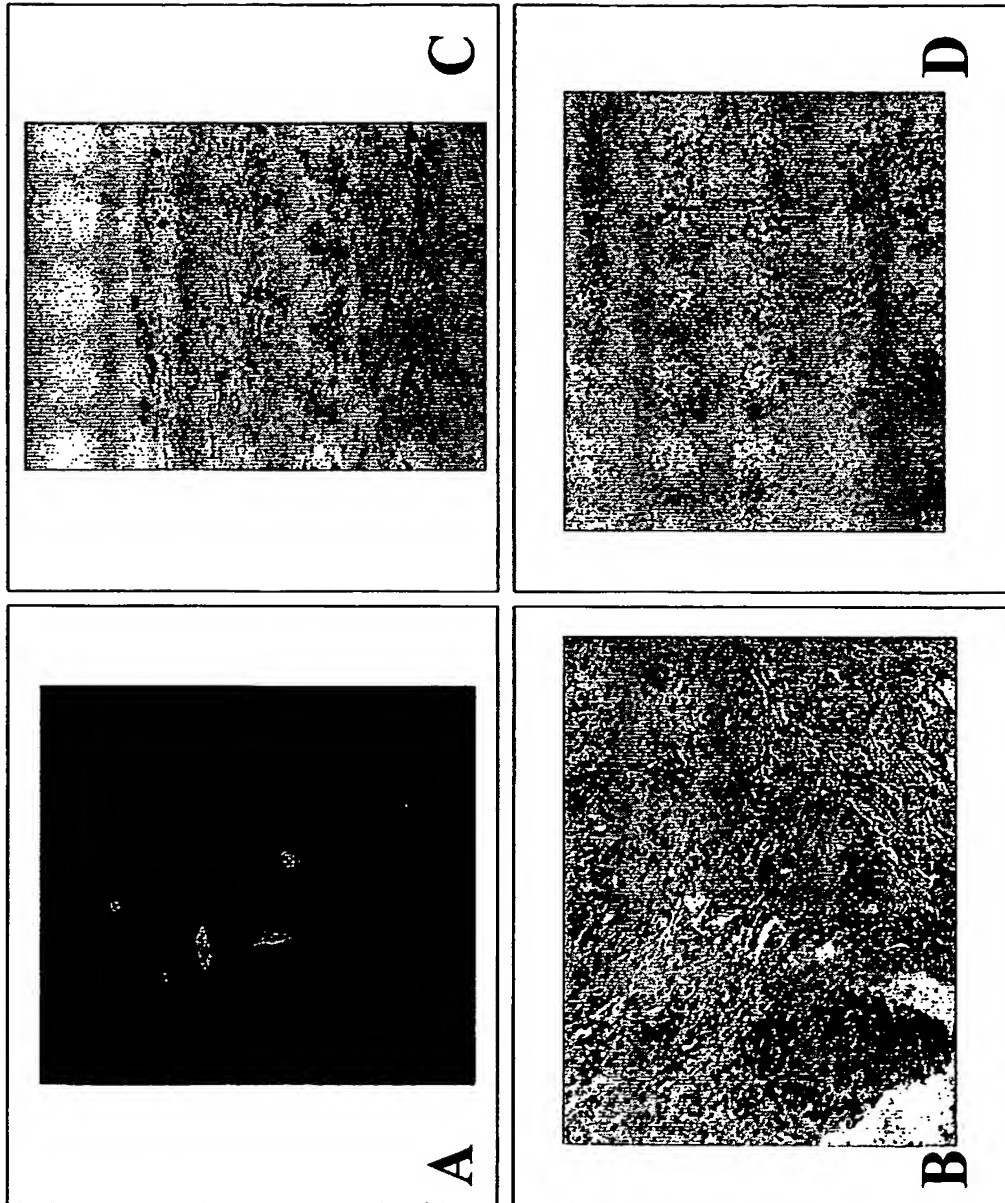


Figure 20B:
ELISA performed with serum from mice (O, L, R, RL, RRL) immunised with
HPV18-E7 protein.



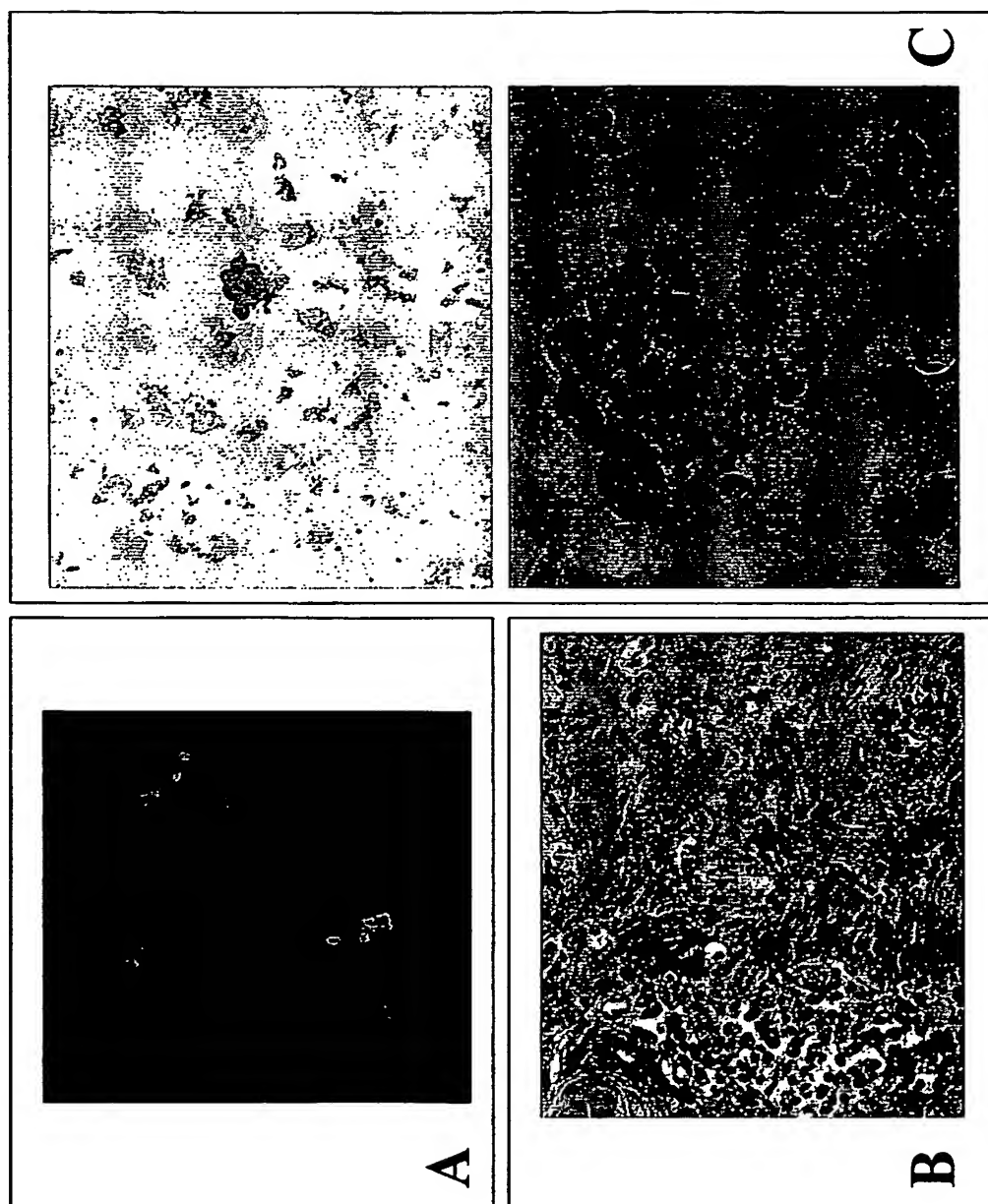
33/37

Figure 21 A:
Immunohistochemically staining of HPV-18 E7 protein by
goat antiHPV-18 E7 antibodies.



BEST AVAILABLE COPY

Figure 21 B :
Immunofluorescence/ Immunohistochemically staining of HPV-45 E7 protein
by a
combination of antiHPV-16 E7 antibodies and antiHPV-18 E7 antibodies.



BEST AVAILABLE COPY

Figure 22 :
Reactivity of antibodies against HPV18 or 16-E7 proteins in IF.
Weak or unspecific signals ○ ; strong and specific signals ●.

	ED17	N19	HyT18	Zym16	RDI16	RDI18	Amy16	Amy18	Amy16 & Amy18
	anti 16E7	anti 18E7	anti 18E7	anti 16E7	Anti 16E7	anti 18E7	anti 16E7	anti 18E7	(combination)
HPV1-E7									
HPV6-E7									
HPV11-E7									
<u>HPV16-E7</u>	○	●	○	○			●		●
<u>HPV18-E7</u>			○		○			●	●
HPV31-E7	○						●		●
HPV33-E7	○						●		●
HPV35-E7		○	○			○	●		●
HPV39-E7		○				○	●		●
HPV45-E7		○	○			○	●		●
HPV52-E7	○						●		●
HPV56-E7		○					●		●
HPV58-E7	○						●		●
HPV59-E7		○					●	●	●

36/37

Figure 23A :
Summary of mABs and pAB tested in W-blot and ELISA

Antibody	against	W-Blot: detection of		ELISA: detection of	
		HPV16-E7	HPV18-E7	HPV16-E7	HPV18-E7
BioCarta Camvir 1	HPV16-L1	not spec. -	not spec. -	-	-
BioCarta cocktail	HPV16,11-16,18-L1	not spec. -	not spec. -	-	-
StaCruz ED17	HPV16-E7	+	-	+	-
StaCruz N19	HPV18-E7	-	+/-	minor signal	minor signal
HyTest 18	HPV18-E7	-	not spec. +	-	+
Zymed 16	HPV16-E7	not spec. +	-	minor signal	-
RDI 16	HPV16-E7	+	-	minor signal	minor signal
RDI 18	HPV18-E7	-	not specific +/-	-	minor signal
Amy 16	HPV16-E7	+	-	++	+
Amy 18	HPV18-E7	-	+	+	++
Combin. A 16&18	HPV16E7 HPV18E7	+	+	++	++

BEST AVAILABLE COPY

37/37

Figure 23B :
Summary of mABs and pAB tested in IF and IHC

Antibody	IF on E7-proteins homologues to		IHC on tumours expressing		
	HPV16 (6 prot.)	HPV18 (5 prot.)	HPV16-E7	HPV18-E7	HPV45-E7
BioCarta Camvir 1	n.d.	n.d.	n.d.	n.d.	n.d.
BioCarta cocktail	n.d.	n.d.	n.d.	n.d.	n.d.
StaCruz ED17	weak (5/6)	-	+	+	+
StaCruz N19	-	weak 4/5 str. 18E7	-	-	-
HyTest 18	-	weak (3/5)	-	1/2 +	-
Zymed 16	weak (1/6)	-	+	-	-
RDI 16	-	-	-	-	-
RDI 18	-	weak (3/5)	-	1/2 +	-
Amy 16	strong (6/6)	strong (4/5)	+	-	+
Amy 18	strong (1/6)	strong (4/5)	-	+	+
Combin. A 16&18	strong (6/6)	strong (5/5)	+	+	+

BEST AVAILABLE COPY